(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 November 2001 (15.11.2001)

PCT

(10) International Publication Number
WO 01/85986 A2

(51) International Patent Classification[7]: C12Q 1/48, A61P 19/00, 29/00, 35/00, 37/00

(21) International Application Number: PCT/US01/15065

(22) International Filing Date: 10 May 2001 (10.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/203,346 10 May 2000 (10.05.2000) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/85986 A2

(54) Title: PHOSPHATIDYL INOSITOL 3-KINASE DELTA BINDING PARTNER

(57) Abstract: There is identified a functional interaction between the catalytic subunit of phosphatidyl inositol 3-kinase delta (P13Kδ) and SH3 domain-containing polypeptides such as LASP-1. The invention provides methods of assaying the observed interaction, methods of exploiting the interaction to identify compounds that modulate the interaction, and methods of employing such modulators in the treatment of medical disorders characterized by P13Kδactivity mediated by the interaction.

PHOSPHATIDYL INOSITOL 3-KINASE DELTA BINDING PARTNER

The present invention relates generally to phosphatidylinositol 3-kinase delta (PI3Kδ) enzyme, and more particularly to binding partners of PI3Kδ, and to methods of using such materials.

BACKGROUND OF THE INVENTION

Cell signaling via 3'-phosphorylated phosphoinositides has been implicated in a variety of cellular processes, e.g., malignant transformation, growth factor signaling, inflammation, and immunity [see Rameh et al., *J Biol Chem* 274:8347-8350 (1999) for a review]. The enzyme responsible for generating these phosphorylated signaling products, phophatidyl inositol 3-kinase (PI 3-kinase; PI3K), was originally identified as an activity associated with viral oncoproteins and growth factor receptor tyrosine kinases that phosphorylates phosphatidyl inositol (PI) and its phosphorylated derivatives at the 3'-hydroxyl of the inositol ring [Panayotou et al., *Trends Cell Biol* 2:358-60 (1992)].

The levels of phophatidyl inositol-3,4,5-triphosphate (PIP_3), the primary product of PI 3-kinase activation, increase upon treatment of cells with a variety of agonists. PI 3-kinase activation is, therefore, believed to be involved in a range of cellular responses including cell growth, differentiation, and apoptosis [Parker et al., *Current Biology* 5:577-99 (1995); Yao et al., *Science* 267:2003-05 (1995)]. Though the downstream targets of phosphorylated lipids generated following PI 3-kinase activation have not been well characterized, emerging evidence suggests that pleckstrin-homology domain- and FYVE-finger domain-containing proteins are activated upon binding to various phosphatidylinositol lipids [Sternmark et al., *J Cell Sci* 112:4175-83 (1999); Lemmon et al., *Trends Cell Biol* 7:237-42 (1997)]. *In vitro*, some isoforms of protein kinase C (PKC) are directly activated by PIP_3, and the PKC-related protein kinase, PKB, has been shown to be activated by PI 3-kinase [Burgering et al., *Nature* 376:599-602 (1995)].

Presently, the PI 3-kinase enzyme family has been divided into three classes based on their substrate specificities. Class I PI3Ks can phosphorylate phosphatidyl inositol (PI), phosphatidyl inositol-4-phosphate, and phosphatidyl inositol-4,5-biphosphate (PIP_2) to produce phosphatidyl inositol-3-phosphate (PIP), phosphatidyl

inositol-3,4-biphosphate, and phosphatidyl inositol-3,4,5-triphosphate, respectively. Class II PI3Ks phosphorylate PI and phosphatidyl inositol-4-phosphate, whereas Class III PI3Ks can only phosphorylate PI.

The initial purification and molecular cloning of PI 3-kinase revealed that it was a heterodimer consisting of p85 and p110 subunits [Otsu et al., *Cell* 65:91-104 (1991); Hiles et al., *Cell* 70:419-29 (1992)]. Since then, four distinct Class I PI3Ks have been identified, designated PI3K α, β, δ, and γ, each consisting of a distinct 110 kDa catalytic subunit and a regulatory subunit. More specifically, three of the catalytic subunits, i.e., p110α, p110β and p110δ, each interact with the same regulatory subunit, p85; whereas p110γ interacts with a distinct regulatory subunit, p101. As described below, the patterns of expression of each of these PI3Ks in human cells and tissues are also distinct. Though a wealth of information has been accumulated in recent past on the cellular functions of PI 3-kinases in general, the roles played by the individual isoforms are largely unknown.

Cloning of bovine p110α was described in Hiles et al. [*supra*]. This protein was identified as related to the *Saccharomyces cerevisiae* protein: Vps34p, a protein involved in vacuolar protein processing [*Id.*]. The recombinant p110α product was also shown to associate with p85α, to yield a PI3K activity in transfected COS-1 cells [Hiles et al., *supra*].

The cloning of a second human p110 isoform, designated p110β, is described by Hu et al. [*Mol Cell Biol* 13:7677-88 (1993)]. This isoform is said to associate with p85 in cells, and to be ubiquitously expressed, as p110β mRNA has been found in numerous human and mouse tissues as well as in human umbilical vein endothelial cells, Jurkat human leukemic T cells, 293 human embryonic kidney cells, mouse 3T3 fibroblasts, HeLa cells, and NBT2 rat bladder carcinoma cells [*Id.*]. Such wide expression suggests that this isoform is broadly important in signaling pathways.

Identification of the p110δ isoform of PI 3-kinase is described in Chantry et al., *J Biol Chem* 272:19236-41 (1997). It was observed that the human p110δ isoform is expressed in a tissue-restricted fashion; it is expressed at high levels in lymphocytes and lymphoid tissues, suggesting that the protein might play a role in PI 3-kinase-mediated signaling in the immune system. Details concerning the P110δ isoform may also be found in U.S. Patent Nos. 5,858,753, 5,822,910, and 5,985,589.

See also, Vanhaesebroeck et al., *Proc Natl Acad Sci USA* 94:4330-5 (1997), and international publication WO 97/46688.

In each of the PI3Kα, β, and δ subtypes, the p85 subunit acts to localize PI 3-kinase to the plasma membrane by the interaction of its SH2 domain with phosphorylated tyrosine residues (present in an appropriate sequence context) in target proteins [Rameh et al., *Cell* 83:821-30 (1995)]. Two isoforms of p85 have been identified, p85α, which is ubiquitously expressed, and p85β, which is primarily found in the brain and lymphoid tissues [Volinia et al., *Oncogene* 7:789-93 (1992)]. Association of the p85 subunit to the PI 3-kinase p110α, β, or δ catalytic subunits appears to be required for the catalytic activity and stability of these enzymes. In addition, the binding of Ras proteins also upregulates PI 3-kinase activity.

The cloning of p110γ revealed still further complexity within the PI3K family of enzymes [Stoyanov et al., *Science* 269:690-93 (1995)]. The p110γ isoform is closely related to p110α and p110β (45-48% identity in the catalytic domain), but as noted does not make use of p85 as a targeting subunit. Instead, p110γ contains an additional domain termed a "pleckstrin homology domain" near its amino terminus. This domain allows interaction of p110γ with the βγ subunits of heterotrimeric G proteins and this interaction appears to regulate its activity [Stoyanov et al., *supra*].

The p101 regulatory subunit for PI3Kgamma was originally cloned in swine, and the human ortholog identified subsequently [Krugmann et al., *J Biol Chem* 274:17152-8 (1999)]. Interaction between the N-terminal region of p101 with the N-terminal region of p110γ appears to be critical for the PI3Kγ activation through Gβγ mentioned above.

A constitutively active PI3K polypeptide is described in international publication WO 96/25488. This document describes preparation of a chimeric fusion protein in which a 102-residue fragment of p85 known as the inter-SH2 (iSH2) region is fused through a linker region to the N-terminus of murine p110. The p85 iSH2 domain apparently is able to activate PI3K activity in a manner comparable to intact p85 [Klippel et al., *Mol Cell Biol* 14:2675-85 (1994)].

Thus, PI 3-kinases may be defined by their amino acid identity or by their activity. Additional members of this growing gene family include more distantly related lipid and protein kinases including Vps34 TOR1, and TOR2 of

Saccharomyces cerevisiae (and their mammalian homologs such as FRAP and mTOR), the ataxia telangiectasia gene product (ATR) and the catalytic subunit of DNA-dependent protein kinase (DNA-PK). See generally, Hunter, *Cell* 83:1-4 (1995).

PI 3-kinase also appears to be involved in a number of aspects of leukocyte activation. A p85-associated PI 3-kinase activity has been shown to physically associate with the cytoplasmic domain of CD28, which is an important costimulatory molecule for the activation of T-cells in response to antigen [Pages et al., *Nature* 369:327-29 (1994); Rudd, *Immunity* 4:527-34 (1996)]. Activation of T cells through CD28 lowers the threshold for activation by antigen and increases the magnitude and duration of the proliferative response. These effects are linked to increases in the transcription of a number of genes including interleukin-2 (IL2), an important T cell growth factor [Fraser et al., *Science* 251:313-16 (1991)]. Mutation of CD28 such that it can no longer interact with PI 3-kinase leads to a failure to initiate IL2 production, suggesting a critical role for PI 3-kinase in T cell activation [Pages et al., *supra*].

Specific inhibitors against individual members of a family of enzymes provide invaluable tools for deciphering function(s) of each enzyme. Two compounds, LY294002 and wortmannin, have been widely used as PI 3-kinase inhibitors. These compounds, however, are non-specific PI3K inhibitors, as they do not distinguish among the four members of Class I PI 3-kinases. For example, the IC_{50}'s of wortmannin against the each of the various Class I PI 3-kinases are in the range of 1-10 nM. Similarly, the IC_{50} for LY294002 against each of these PI 3-kinases is about 1 μM [Fruman et al., *Ann Rev Biochem* 67:481-507 (1998)]. Hence, the utility of these compounds in studying the roles of individual Class I PI 3-kinases is limited.

Based on studies using the wortmannin, there is evidence that PI 3-kinase function is also required for some aspects of leukocyte signaling through G-protein coupled receptors [Thelen et al., *Proc Natl Acad Sci USA* 91:4960-64 (1994)]. Moreover, it has been shown that wortmannin and LY294002 block neutrophil migration and superoxide release. However, inasmuch as these compounds do not distinguish among the various isoforms of PI3K, it remains unclear which particular PI3K isoform or isoforms are involved in these phenomena.

In view of the above considerations, it is clear that existing knowledge is lacking with respect to structural and functional features of the PI 3-kinase enzymes, including sub-cellular localization, activation states, substrate affinities, and the like. Moreover, the functions that these enzymes perform in both normal and diseased tissues remains to be elucidated. In particular, the function of PI3Kδ in leukocytes has not previously been characterized, and knowledge concerning its function in human physiology remains limited. The coexpression in these tissues of other PI3K isoforms has heretofore confounded efforts to segregate the activities of each enzyme. Furthermore, separation of the activities of the various PI3K isozymes may not be possible without identification of inhibitors that demonstrate selective inhibition characteristics.

Thus, there exists a need in the art for further structural and functional characterization of the PI3Kδ enzyme. Furthermore, our understanding of PI3Kδ requires further elaboration of the structural interactions of p110δ, both with its regulatory subunit and with other proteins in the cell. There also remains a need for selective or specific inhibitors of PI3K isozymes, in order that the functions and structural interactions of each isozyme may be better characterized.

One of the purposes of the present invention is to provide methods for identifying compounds that can inhibit PI3Kδ activity, and preferably compounds that inhibit interaction of PI3Kδ with its binding partners. Another purpose of the invention is to provide compounds that inhibit PI3Kδ selectively while having relatively low inhibitory potency against the other PI3K isoforms. Another purpose of the invention is to provide methods of characterizing the function of human PI3Kδ. Another purpose of the invention is to provide methods of selectively modulating human PI3Kδ activity, and thereby to promote medical treatment of diseases mediated by PI3Kδ dysfunction. Other purposes and advantages of the invention will be readily apparent to the artisan having ordinary skill in the art.

SUMMARY OF THE INVENTION

It has now been discovered that these and other purposes can be achieved by the present invention, which, in one aspect, is a method of identifying a modulator of p110δ binding to LASP-1, comprising the steps of:

(a) providing a p110δ polypeptide and a LASP-1 polypeptide having specific binding affinity for one another;

(b) measuring binding between the p110δ polypeptide and the LASP-1 polypeptide in the presence and absence of a test compound; and

(c) identifying the test compound as a modulator of p110δ binding to LASP-1 when a different amount of binding of the p110δ polypeptide to the LASP-1 polypeptide is detected in the presence of the test compound than in the absence of the test compound.

The p110δ polypeptide and the LASP-1 polypeptide can be provided in a system free of cellular components, or may be recombinantly expressed or coexpressed in host cells. In some embodiments, the p110δ polypeptide comprises the proline rich region of p110δ. Likewise, in some embodiments, the LASP-1 polypeptide comprises the SH3 region of LASP-1. Either or both of the polypeptides may be provided as fusion proteins to impart desirable properties to the polypeptides, e.g., to permit secretion or cell surface expression of the polypeptides. The method may be employed to identify modulators that inhibit binding of p110δ to LASP-1 or modulators that enhance binding of p110δ to LASP-1.

In another aspect, the invention is a compound having activity as a modulator of interaction between p110δ and LASP-1, wherein the compound is identified according to the method described herein. For example, the compound may be a compound that inhibits binding of p110δ to LASP-1, or a compound that enhances binding of p110δ to LASP-1.

In another aspect, the invention is a method of treating a disease state characterized by undesirable or excessive activity of PI3Kδ, comprising administering to a subject in need thereof a compound that modulates interaction of p110δ with LASP-1 in an amount effective to modulate interaction of p110δ with LASP-1. Preferably, the compound inhibits p110δ:LASP-1 interaction. Compounds

suitable for use in this method may be identified using screening methods described herein, or may be derived analogs thereof.

In still another aspect, the invention is a method of modulating p110δ binding to LASP-1, comprising the step of contacting p110δ or LASP-1 with a modulator of p110δ binding to LASP-1. Preferably, the method employs a modulator that inhibits binding of p110δ to LASP-1.

In yet another aspect, the invention is a method of treating a disease state associated with p110δ binding to LASP-1, comprising the step of administering to a subject in need thereof an effective amount of a modulator of p110δ binding to LASP-1. It is preferred that the method employs a modulator that inhibits binding of p110δ to LASP-1.

As noted in the examples herein, LASP-1 comprises an SH3 domain that interacts with the proline rich sequence of p110δ (aa 288-314 of SEQ ID NO: 2). This proline rich sequence is contemplated as a site of interaction with other polypeptides comprising one or more SH3 domains, for example, Src family kinases, adapter proteins vav and cbl, the p47-phox component of NADPH oxidase, Bruton's tyrosine kinase (Btk) and the p85 component of PI3 kinase. Therefore, in another aspect, the invention is a method of identifying a modulator of p110δ binding to a polypeptide comprising an SH3 domain, comprising the steps of:

(a) providing a p110δ polypeptide and an SH3 domain-containing polypeptide having specific binding affinity for one another;

(b) measuring binding between the p110δ polypeptide and the SH3 domain-containing polypeptide in the presence and absence of a test compound; and

(c) identifying the test compound as a modulator of p110δ binding to the SH3-domain containing polypeptide when a different amount of binding of the p110δ polypeptide to the SH3 domain-containing polypeptide is detected in the presence of the test compound than in the absence of the test compound.

The p110δ polypeptide and the SH3 domain-containing polypeptide can be provided in a system free of cellular components, or may be recombinantly expressed or coexpressed in host cells. In these embodiments, the p110δ polypeptide comprises the proline rich region of p110δ. Either or both of the polypeptides may be provided

as fusion proteins to impart desirable properties to the polypeptides, e.g., to permit secretion or cell surface expression of the polypeptides. The method may be employed to identify modulators that inhibit binding of p110δ to SH3 domain-containing polypeptides or modulators that enhance binding of p110δ to SH3 domain-containing polypeptides.

In another aspect, the invention is a compound having activity as a modulator of interaction between p110δ and an SH3 domain-containing polypeptide, wherein the compound is identified according to the method described herein. For example, the compound may be a compound that inhibits binding of p110δ to an SH3 domain-containing polypeptide, or a compound that enhances binding of p110δ to an SH3 domain-containing polypeptide.

In another aspect, the invention is a method of treating a disease state characterized by undesirable or excessive activity of PI3Kδ, comprising administering to a subject in need thereof a compound that modulates interaction of p110δ with an SH3 domain-containing polypeptide in an amount effective to modulate interaction of p110δ with an SH3 domain-containing polypeptide. Preferably, the compound inhibits p110δ:SH3 domain-containing polypeptide interaction. Compounds suitable for use in this method may be identified using screening methods described herein, or may be derived analogs thereof.

In still another aspect, the invention is a method of modulating p110δ binding to an SH3 domain-containing polypeptide, comprising the step of contacting p110δ or the SH3 domain-containing polypeptide with a modulator of p110δ binding to the SH3 domain-containing polypeptide. Preferably, the method employs a modulator that inhibits binding of p110δ to the SH3 domain-containing polypeptide.

In yet another aspect, the invention is a method of treating a disease state associated with p110δ binding to an SH3 domain-containing polypeptide, comprising the step of administering to a subject in need thereof an effective amount of a modulator of p110δ binding to the SH3 domain-containing polypeptide. It is preferred that the method employs a modulator that inhibits binding of p110δ to the SH3 domain-containing polypeptide.

These and other features and advantages of the present invention will be appreciated from the detailed description and examples that are set forth herein. The detailed description and examples are provided to enhance the understanding of the invention, but are not intended to limit the scope of the invention. In particular, while the following detailed description and examples focus on the interaction of p110δ and LASP-1, the detailed description and examples are intended to be illustrative of interaction of p110δ with other polypeptides comprising SH3 domains. Thus, for example, in the detailed description below discussion of LASP-1 is applicable to SH3 domain-containing polypeptides such as Src family kinases, adapter proteins vav and cbl, the p47-phox component of NADPH oxidase, Bruton's tyrosine kinase (Btk) and the p85 component of PI3 kinase.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention provides methods of characterizing an interaction between the p110δ subunit of phosphatidyl inositol 3-kinase delta (PI3Kδ), i.e., p110δ, and LASP-1. There are further provided methods of identifying modulators, e.g., inhibitors and enhancers, of p110δ:LASP-1 binding. Also provided are methods of employing modulators of PI3Kδ activity associated with or mediated by p110δ:LASP-1 binding, including methods of modulating the activity of the PI3Kδ isozyme in cells, especially leukocytes, osteoclasts, and cancer cells. The methods include *in vitro, in vivo*, or *ex vivo* applications. Of particular benefit are methods of selectively modulating p110δ:LASP-1 interactions in the clinical setting, to treat disease states mediated by PI3Kδ activity. Thus, disease states characterized by excessive or inappropriate PI3Kδ activity may be treated through use of selective modulators of p110δ:LASP-1 binding according to the invention. Other methods enabled by the invention include methods necessary for further characterization of the p110δ:LASP-1 interaction *in vivo*. Details of these and other useful embodiments of the invention are now described.

The methods described herein benefit from the use of compounds that selectively inhibit p110δ:LASP-1 interaction in cells *in vitro, in vivo*, or *ex vivo*. Cells useful in the methods include those that express endogenous p110δ and LASP-1. By "endogenous" it is meant that the cells express p110δ and/or LASP-1 absent

recombinant introduction into the cells of one or more polynucleotides encoding the polypeptide(s) or biologically active fragments thereof. Methods also encompass use of host cells that have been modified recombinantly to express exogenous p110δ and/or LASP-1, wherein one or more polynucleotides encoding p110δ or LASP-1, or biologically active fragments thereof, have been introduced into the cell using recombinant procedures. Host cells coexpressing a p110δ polypeptide and a LASP-1 polypeptide are provided, as are recombinant expression vectors enabling such coexpression, e.g., vectors comprising DNAs encoding the polypeptides arranged in tandem. Of particular advantage, the cells may be *in vivo*, i.e., in a living subject, e.g., an animal or human, wherein a modulator of p110δ:LASP-1 binding may be used as a therapeutic to modulate PI3Kδ activity in the subject. Alternatively, the cells may be isolated as discrete cells or in a tissue, for *ex vivo* or *in vitro* methods.

In vitro methods also comprehended by the invention can comprise the step of contacting an isolated p110δ polypeptide or LASP-1 polypeptide with a modulator of p110δ:LASP-1 binding. In such "biochemical" methods, the p110δ and LASP-1 polypeptides are provided as purified and isolated polypeptides, i.e., the polypeptides are isolated from a natural source (e.g., cells or tissues that normally express at least one of the polypeptides absent modification by recombinant technology) or isolated from cells modified by recombinant techniques to express at least one of the polypeptides.

Compounds of any type that selectively modulate p110δ:LASP-1 binding may be used as modulators in the methods of the invention. Moreover, compounds of any type that modulate, preferably inhibit, p110δ:LASP-1 binding and that possess acceptable pharmacological properties may be used as modulators in the therapeutic methods of the invention.

The relative efficacies of compounds as modulators of a biological activity may be established by determining the concentrations at which each compound affects the activity to a predefined extent and then comparing the results. Typically, the preferred determination is the concentration that inhibits 50% of the activity in a biochemical assay, i.e., the 50% inhibitory concentration or "IC_{50}." IC_{50} determinations may be accomplished using conventional techniques known in the art. In general, IC_{50} may be determined by measuring the given biological activity in the

presence of a range of concentrations of the inhibitor under study. The experimentally obtained values of enzyme activity are then plotted against the inhibitor concentrations used. The concentration of the inhibitor that shows 50% activity (as compared to the activity in the absence of any inhibitor) is taken as the IC_{50}. Analogously, other inhibitory concentrations may be defined through appropriate determinations of activity. For example, in some settings it may be desirable to establish a 90% inhibitory concentration, i.e., IC_{90}, etc.

Methods for Identifying Modulators of p110δ:LASP-1 Binding

The p110δ and LASP-1 polypeptides, as well as fragments thereof possessing biological activity, can be used for screening putative modulator compounds in any of a variety of drug screening techniques. A modulator of p110δ:LASP-1 binding is a compound that increases or decreases the ability of p110δ and LASP-1 to interact with one another, with the consequence of affecting the ability of p110δ to carry out any of its biological functions. An example of such a compound is an agent that, through affecting p110δ:LASP-1 binding, increases or decreases the ability of PI3Kδ to phosphorylate phosphatidyl inositol or to target to appropriate structures within a cell.

The selectivity of a compound that modulates p110δ:LASP-1 binding can be evaluated by comparing its activity on p110δ:LASP-1 binding to its activity on other related proteins or the interactions on other related binding pairs of proteins. To illustrate, selective modulators of p110δ:LASP-1 binding may include, for example, antibodies and other proteins or peptides that specifically bind to a p110δ polypeptide or a LASP-1 polypeptide, oligonucleotides that specifically bind to a p110δ polypeptide or a LASP-1 polypeptide, and other non-peptide compounds (e.g., isolated or synthetic organic molecules) that specifically interact with a p110δ polypeptide or a LASP-1 polypeptide.

Accordingly, the invention provides methods of characterizing the potency of a test compound as a modulator of p110δ:LASP-1 binding, said method comprising the steps of (a) measuring activity of a PI3Kδ polypeptide in the presence of a test compound; (b) comparing the activity of the PI3Kδ polypeptide in the presence of the

test compound to the activity of the PI3Kδ polypeptide in the presence of an equivalent amount of a reference compound; wherein a lower activity of the PI3Kδ polypeptide in the presence of the test compound than in the presence of the reference indicates that the test compound is a more potent inhibitor than the reference compound, and a higher activity of the PI3Kδ polypeptide in the presence of the test compound than in the presence of the reference indicates that the test compound is a less potent inhibitor than the reference compound.

The invention further provides methods of characterizing the potency of a test compound as an inhibitor of p110δ:LASP-1 binding, comprising the steps of (a) determining an amount of a control compound that inhibits p110δ:LASP-1 binding by a reference percentage of inhibition, thereby defining a reference inhibitory amount for the control compound; (b) determining an amount of a test compound that inhibits p110δ:LASP-1 binding by a reference percentage of inhibition, thereby defining a reference inhibitory amount for the test compound; (c) comparing the reference inhibitory amount for the test compound to the reference inhibitory amount for the control compound, wherein a lower reference inhibitory amount for the test compound than for the control compound indicates that the test compound is a more potent inhibitor than the control compound, and a higher reference inhibitory amount for the test compound than for the control compound indicates that the test compound is a less potent inhibitor than the control compound. In one aspect, the method uses a reference inhibitory amount that is the amount of the compound than inhibits p110δ:LASP-1 binding by 50%, 60%, 70%, 80%. In another aspect, the method employs a reference inhibitory amount that is the amount of the compound that inhibits p110δ:LASP-1 binding by 90%, 95%, or 99%. These methods may comprise determining the reference inhibitory amount of the compounds in an *in vitro* biochemical assay, in an *in vitro* cell-based assay, or in an *in vivo* assay.

The invention therefore provides a method for screening for candidate modulators of PI3Kδ activity and/or to confirm the mode of action of candidate such negative regulators, i.e., to determine whether such compound operate through modulation of p110δ:LASP-1 binding. Such methods may be employed against other p110 isoforms (e.g., p110α, p110β, and p110γ) in parallel to establish comparative activity of the test compound across the isoforms.

In these methods, the p110δ polypeptide may be a full-length p110δ polypeptide, e.g., human p110δ having the sequence set forth in SEQ ID NO:2, or it may be a p110δ fragment, provided that the fragment exhibits binding activity for LASP-1. An exemplary p110δ polypeptide is a fragment of p110δ comprising the proline-rich region that has been identified hereunder as interacting with LASP-1. The p110δ fragment may further comprise the catalytic site of p110δ and/or the p85 binding domain of p110δ.

Likewise, the LASP-1 polypeptide may be a full-length LASP-1 polypeptide, e.g., human LASP-1 having the sequence set forth in SEQ ID NO:4, or it may be a LASP-1 fragment, provided that the fragment exhibits binding activity for p110δ. An exemplary LASP-1 polypeptide is a fragment of LASP-1 comprising the SH3 region that has been identified hereunder as interacting with p110δ. The LASP-1 polypeptide may further comprise other domains of the LASP-1 protein, e.g., the LIM region.

The methods may be employed in cells expressing cells expressing p110δ or fragments thereof, either endogenously or exogenously. Accordingly, the polypeptide employed in such methods may be free in solution, affixed to a solid support, modified to be displayed on a cell surface (e.g., as a fusion protein), or located intracellularly. The modulation of activity or the formation of binding complexes between the p110δ polypeptide, the LASP-1 polypeptide, and the agent being tested may then be measured.

LASP-1 and p110δ polypeptides, and the interactions thereof, are amenable to biochemical or cell-based high throughput screening (HTS) assays according to methods known and practiced in the art, including melanophore assay systems to investigate receptor-ligand interactions, yeast-based assay systems, and mammalian cell expression systems. For a review, see Jayawickreme and Kost, *Curr Opin Biotechnol* 8:629-34 (1997). Automated and miniaturized HTS assays are also comprehended as described, for example, in Houston and Banks, *Curr Opin Biotechnol* 8:734-40 (1997).

Such HTS assays are used to screen libraries of compounds to identify particular compounds that exhibit a desired property. Any library of compounds may be used, including chemical libraries, natural product libraries, and combinatorial

libraries comprising random or designed oligopeptides, oligonucleotides, or other organic compounds.

Chemical libraries may contain known compounds, proprietary structural analogs of known compounds, or compounds that are identified from natural product screening.

Natural product libraries are collections of materials isolated from naturals sources, typically, microorganisms, animals, plants, or marine organisms. Natural products are isolated from their sources by fermentation of microorganisms followed by isolation and extraction of the fermentation broths or by direct extraction from the microorganisms or tissues (plants or animal) themselves. Natural product libraries include polyketides, non-ribosomal peptides, and variants (including non-naturally occurring variants) thereof. For a review, see Cane et al., *Science* 282:63-68 (1998).

Combinatorial libraries are composed of large numbers of related compounds, such as peptides, oligonucleotides, or other organic compounds as a mixture. Such compounds are relatively straightforward to design and prepare by traditional automated synthesis protocols, PCR, cloning or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries.

Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created thereby, see Myers, *Curr Opin Biotechnol* 8:701-07 (1997).

Once compounds have been identified that show activity as modulators of p110δ:LASP-1 binding (i.e., "hits"), a program of optimization can be undertaken in an effort to improve the potency and/or selectivity of the activity. Analysis of structure-activity relationships (SAR) typically involves of iterative series of selective modifications of compound structures and their correlation to biochemical or biological activity. Families of related compounds can be designed that all exhibit the desired activity, with certain members of the family, namely those possessing suitable pharmacological profiles, potentially qualifying as therapeutic candidates. Compounds obtained using such medicinal chemistry methods may be referred to a "derived analogs" of the original "hit" compounds.

Related to methods of identifying modulators of p110δ:LASP-1 binding, the invention enables tools that facilitate such methods. For example, containers can be manufactured that contain a p110δ polypeptide and a LASP-1 polypeptide. To illustrate, the polypeptides may be provided in a kit, such as a kit in which the polypeptides are contained in separate containers, and optionally further comprising an instruction sheet providing guidance on performance of the assay. The contents can then be employed for measuring the p110δ:LASP-1 interaction in a modulator identification assay. For HTS assays, the invention provides multi-well (e.g., 96- or 384-well) plates in which at least one well contains a p110δ polypeptide and a LASP-1 polypeptide in amounts suitable for screening small quantities of a test compound. Alternatively, for cell-based assays, the invention provides containers (e.g., multi-well plates) containing host cells recombinantly modified to express, preferably cells modified to coexpress, a p110δ polypeptide and a LASP-1 polypeptide.

Therapeutic Uses of Inhibitors of p110δ:LASP-1 Binding

As described herein, the invention provides a method for identifying compounds capable of selectively modulating p110δ:LASP-1 binding, or modulating PI3Kδ activity mediated thereby. Thus, the invention may provide the basis for development of methods of treatment of PI3Kδ-mediated disease states. Such treatment methods can include administering an modulator, most probably an inhibitor, of p110δ:LASP-1 binding in an amount effective for this purpose. These methods may be employed in treating humans or animals that are or may be subject to any disease state whose symptoms or pathology is characterized by PI3Kδ activity as it may be mediated by p110δ:LASP-1 binding. Among other things, as PI3Kδ expression is predominantly limited to leukocytes, the invention enables methods of inhibiting PI3Kδ function in these cells. Thus, disease states in which leukocyte function is excessive or undesirable may be amenable to treatment using p110δ:LASP-1 binding modulators as described hereinbelow in greater detail.

In one aspect, the invention provides methods of treating disease states characterized by functions of neutrophils. It has been observed that inhibition of PI3Kδ activity inhibits certain functions of neutrophils such as stimulated superoxide release, stimulated exocytosis, and chemotactic migration. Superoxide is released by neutrophils in response to any of a variety of stimuli including signals of infection, as a mechanism of cell killing. For example, superoxide release is known to be induced by tumor necrosis factor alpha (TNFα), which is released by macrophages, mast cells, and lymphocytes upon contact with bacterial cell wall components such as lipopolysaccharide (LPS). TNFα is an extraordinarily potent and promiscuous activator of inflammatory processes, being involved in activation of neutrophils and various other cell types, induction of leukocyte/endothelial cell adhesion, pyrexia, enhanced MHC class I production, and stimulation of angiogenesis. Alternatively, superoxide release may be stimulated by formyl-Met-Leu-Phe (fMLP) or other peptides blocked at the N-terminus by formylated methionine. Such peptides are not normally found in eukaryotes, but are fundamentally characteristic of bacteria, and signal the presence of bacteria to the immune system. Leukocytes expressing the fMLP receptor, e.g., neutrophils and macrophages, are stimulated to migrate up

gradients of these peptides (i.e., chemotaxis) toward loci of infection. In general, these functions may be collectively termed "inflammatory functions," as they are typically related to neutrophil response to inflammation. Such functions may further include, without limitation, stimulated degranulation, adhesion to vascular endothelium (e.g., tethering/rolling of neutrophils, triggering of neutrophil activity, and/or latching of neutrophils to endothelium), transmural diapedesis or emigration through the endothelium to peripheral tissues. The inflammatory functions of neutrophils may be distinguished from the bacterial killing functions exhibited by these cells, e.g., phagocytosis and killing of bacteria.

Without intending to be bound by any one theory, it is believed that, because inflammation involves processes are typically mediated by leukocyte (e.g., neutrophil, lymphocyte, etc.) activation and chemotactic transmigration, and because PI3Kδ may mediate such phenomena, antagonists of PI3Kδ activity mediated by p110δ:LASP-1 binding may be used to suppress injury associated with inflammation. Accordingly, the invention further includes methods of treating disease states in which one or more of the inflammatory functions of neutrophils are abnormal or undesirable. Neutrophil functions suitable for inhibition according to the method include any function that is characterized by PI3Kδ activity mediated by p110δ:LASP-1 binding.

It has further been observed that PI3Kδ plays a role in the stimulated proliferation of lymphocytes, including B cells and T cells. Moreover, PI3Kδ appears to play a role in stimulated secretion of antibodies by B cells. Thus, the invention includes methods for inhibiting lymphocyte proliferation, and methods for inhibiting antibody production by B lymphocytes through the use of modulators of p110δ:LASP-1 binding. Other methods enabled by the invention include methods of treating disease states in which one or more of these lymphocyte functions are abnormal or undesirable.

In another aspect, the invention includes a method for suppressing a function of basophils and/or mast cells, and thereby enabling treatment of disease states characterized by excessive or undesirable basophil and/or mast cell activity. According to the method, a compound of the invention may be used that selectively inhibits p110δ:LASP-1 binding and associated activity of phosphatidylinositol 3-kinase delta (PI3Kδ) in basophils and/or mast cells. Preferably, the method employs

a p110δ:LASP-1 inhibitor in an amount sufficient to inhibit stimulated histamine release by the basophils and/or mast cells. Accordingly, the use of such compounds and other PI3Kδ selective inhibitors may be of value in treating diseases characterized by histamine release, i.e., allergic disorders, including disorders such as chronic obstructive pulmonary disease (COPD), asthma, ARDS, emphysema, and related disorders.

In another aspect, selective inhibitors of PI3Kδ activity mediated by p110δ:LASP-1 binding may be employed in methods of treating diseases of bone, especially diseases in which osteoclast function is abnormal or undesirable. Accordingly, the use of such compounds may be of value in treating osteoporosis, Paget's disease, and related bone resorption disorders.

In a further aspect, the invention includes methods of using modulators of p110δ:LASP-1 binding to inhibit the growth or proliferation of cancer cells of hematopoietic origin, preferably cancer cells of lymphoid origin, and more preferably cancer cells related to or derived from B lymphocytes or B lymphocyte progenitors. Cancers potentially amenable to treatment using the method of the invention include, without limitation, lymphomas, i.e., malignant neoplasms of lymphoid and reticuloendothelial tissues, such as Burkitt's lymphoma, Hodgkins' lymphoma, non-Hodgkins lymphomas, lymphocytic lymphomas and the like; multiple myelomas; as well as leukemias such as lymphocytic leukemias, chronic myeloid (myelogenous) leukemias, and the like. In a preferred embodiment, p110δ:LASP-1 modulatory compounds may be used to inhibit or control the growth or proliferation of chronic myeloid (myelogenous) leukemia cells.

"Treating" as used herein refers to preventing a disease state from occurring in an animal that may be predisposed to the disease state, but has not yet been diagnosed as having it; inhibiting the disease state, i.e., arresting its development; relieving the disease state, i.e., causing its regression; or ameliorating the disease state, i.e., reducing the severity of symptoms associated with the disease state.

"Disease state" as used herein is intended to encompass pathological disorders, diseases, conditions, syndromes, and the like, without limitation.

"Inflammatory disease" as used herein can refer to any disease state in which an excessive or unregulated inflammatory response leads to excessive inflammatory

symptoms, host tissue damage, or loss of tissue function. "Inflammatory disease" also refers to a pathological state mediated by influx of leukocytes and/or neutrophil chemotaxis.

"Inflammation" as used herein refers to a localized, protective response elicited by injury or destruction of tissues, which serves to destroy, dilute, or wall off (sequester) both the injurious agent and the injured tissue. Inflammation is notably associated with influx of leukocytes and/or neutrophil chemotaxis. Inflammation may result from infection with pathogenic organisms and viruses and from noninfectious means such as trauma or reperfusion following myocardial infarction or stroke, immune response to foreign antigen, and autoimmune responses. Accordingly, inflammatory diseases amenable to the invention encompass disease states associated with reactions of the specific defense system as well as with reactions of the non-specific defense system.

"Specific defense system" as used herein refers to the component of the immune system that reacts to the presence of specific antigens. Examples of inflammation resulting from a response of the specific defense system include the classical response to foreign antigens, autoimmune diseases, and delayed type hypersensitivity response mediated by T-cells. Chronic inflammatory diseases, the rejection of solid transplanted tissue and organs, e.g., kidney and bone marrow transplants, and graft versus host disease (GVHD), are further examples of inflammatory reactions of the specific defense system.

"Non-specific defense system" as used herein refers to the cells systems involved in inflammatory processes that are mediated by leukocytes that are incapable of immunological memory (e.g., granulocytes, and macrophages). Examples of inflammation that result, at least in part, from a reaction of the non-specific defense system include inflammation associated with conditions such as adult (acute) respiratory distress syndrome (ARDS) or multiple organ injury syndromes; reperfusion injury; acute glomerulonephritis; reactive arthritis; dermatoses with acute inflammatory components; acute purulent meningitis or other central nervous system inflammatory diseases such as stroke; thermal injury; inflammatory bowel disease; granulocyte transfusion associated syndromes; and cytokine-induced toxicity.

"Autoimmune disease" as used herein refers to any group of disease states in which tissue injury is associated with humoral or cellmediated responses to the body's own constituents. "Allergic disease" as used herein refers to any disease state in which symptoms, tissue damage, or loss of tissue function result from allergy. "Arthritic disease" as used herein refers to any disease state that is characterized by inflammatory lesions of the joints attributable to a variety of etiologies. "Dermatitis" as used herein refers to any of a large family of diseases of the skin that are characterized by inflammation of the skin attributable to a variety of etiologies. "Transplant rejection" as used herein refers to any immune reaction directed against grafted tissue, such as organs or cells (e.g., bone marrow), characterized by a loss of function of the grafted and surrounding tissues, pain, swelling, leukocytosis, and thrombocytopenia.

The therapeutic methods of the present invention include methods for the treatment of disease states associated with inflammatory cell activation. "Inflammatory cell activation" refers to the induction by a stimulus (including, but not limited to, cytokines, antigens or autoantibodies) of a proliferative cellular response, the production of soluble mediators (including but not limited to cytokines, oxygen radicals, enzymes, prostanoids, or vasoactive amines), or cell surface expression of new or increased numbers of mediators (including, but not limited to, major histocompatability antigens or cell adhesion molecules) in inflammatory cells (including but not limited to monocytes, macrophages, T lymphocytes, B lymphocytes, granulocytes (i.e., polymorphonuclear leukocytes such as neutrophils, basophils, and eosinophils), mast cells, dendritic cells, Langerhans cells, and endothelial cells). It will be appreciated by persons skilled in the art that the activation of one or a combination of these phenotypes in these cells can contribute to the initiation, perpetuation, or exacerbation of an inflammatory disease.

The present invention enables methods of treating such diseases as arthritic diseases, such as rheumatoid arthritis, monoarticular arthritis, osteoarthritis, gouty arthritis, spondylitis; Behcet disease; sepsis, septic shock, endotoxic shock, gram negative sepsis, gram positive sepsis, and toxic shock syndrome; multiple organ injury syndrome secondary to septicemia, trauma, or hemorrhage; ophthalmic disorders such as allergic conjunctivitis, vernal conjunctivitis, uveitis, and thyroid-

associated ophthalmopathy; eosinophilic granuloma; pulmonary or respiratory disorders such as asthma, chronic bronchitis, allergic rhinitis, ARDS, chronic pulmonary inflammatory disease (e.g., chronic obstructive pulmonary disease), silicosis, pulmonary sarcoidosis, pleurisy, alveolitis, vasculitis, emphysema, pneumonia, bronchiectasis, and pulmonary oxygen toxicity; reperfusion injury of the myocardium, brain, or extremities; fibrosis such as cystic fibrosis; keloid formation or scar tissue formation; atherosclerosis; autoimmune diseases such as systemic lupus erythematosus (SLE), autoimmune thyroiditis, multiple sclerosis, some forms of diabetes, and Reynaud's syndrome; and transplant rejection disorders such as GVHD and allograft rejection; chronic glomerulonephritis; inflammatory bowel diseases such as chronic inflammatory bowel disease (CIBD), Crohn's disease, ulcerative colitis, and necrotizing enterocolitis; inflammatory dermatoses such as contact dermatitis, atopic dermatitis, psoriasis, or urticaria; fever and myalgias due to infection; central or peripheral nervous system inflammatory disorders such as meningitis, encephalitis, and brain or spinal cord injury due to minor trauma; Sjögren's syndrome; diseases involving leukocyte diapedesis; alcoholic hepatitis; bacterial pneumonia; antigen-antibody complex mediated diseases; hypovolemic shock; Type I diabetes mellitus; acute and delayed hypersensitivity; disease states due to leukocyte dyscrasia and metastasis; thermal injury; granulocyte transfusion-associated syndromes; and cytokine-induced toxicity.

The method can have utility in treating subjects that are or may be subject to reperfusion injury, i.e., injury resulting from situations in which a tissue or organ experiences a period of ischemia followed by reperfusion. The term "ischemia" refers to localized tissue anemia due to obstruction of the inflow of arterial blood. Transient ischemia followed by reperfusion characteristically results in neutrophil activation and transmigration through the endothelium of the blood vessels in the affected area. Accumulation of activated neutrophils in turn results in generation of reactive oxygen metabolites, which damage components of the involved tissue or organ. This phenomenon of "reperfusion injury" is commonly associated with conditions such as vascular stroke (including global and focal ischemia), hemorrhagic shock, myocardial ischemia or infarction, organ transplantation, and cerebral vasospasm. To illustrate, reperfusion injury occurs at the termination of cardiac

bypass procedures or during cardiac arrest when the heart, once prevented from receiving blood, begins to reperfuse. It is expected that inhibition of PI3Kδ activity will result in reduced amounts of reperfusion injury in such situations.

With respect to the nervous system, global ischemia occurs when blood flow to the entire brain ceases for a period. Global ischemia may result from cardiac arrest. Focal ischemia occurs when a portion of the brain is deprived of its normal blood supply. Focal ischemia may result from thromboembolic occlusion of a cerebral vessel, traumatic head injury, edema, or brain tumor. Even if transient, both global and focal ischemia can cause widespread neuronal damage. Although nerve tissue damage occurs over hours or even days following the onset of ischemia, some permanent nerve tissue damage may develop in the initial minutes following the cessation of blood flow to the brain.

Ischemia can also occur in the heart in myocardial infarction and other cardiovascular disorders in which the coronary arteries have been obstructed as a result of atherosclerosis, thrombus, or spasm. Accordingly, the invention is believed to be useful for treating cardiac tissue damage, particularly damage resulting from cardiac ischemia or caused by reperfusion injury in mammals.

The methods of the invention embrace various modes of treating an animal, preferably a mammal, more preferably a primate, and still more preferably a human. Among the mammals that may be treated are, for example, companion animals (pets) including dogs and cats; farm animals including cattle, horses, sheep, pigs, and goats; laboratory animals including rats, mice, rabbits, guinea pigs, and non-human primates. Non-mammalian animals include, for example, birds, fish, reptiles, and amphibians.

Methods of Administration of Modulators of p110δ:LASP-1 Interaction

Pharmaceutical compositions comprising a modulator of p110δ:LASP-1 binding activity may be administered to the subject by any conventional method, including parenteral and enteral techniques. Parenteral administration modalities include those in which the composition is administered by a route other than through the gastrointestinal tract, for example, intravenous, intraarterial, intraperitoneal, intramedullary, intramuscular, intraarticular, intrathecal, and intraventricular injections. Enteral administration modalities include, for example, oral (including buccal and sublingual) and rectal administration. Transepithelial administration modalities include, for example, transmucosal administration and transdermal administration. Transmucosal administration includes, for example, enteral administration as well as nasal, inhalation, and deep lung administration; vaginal administration; and rectal administration. Transdermal administration includes passive or active transdermal or transcutaneous modalities, including, for example, patches and iontophoresis devices, as well as topical application of pastes, salves, or ointments. Parenteral administration can also be accomplished using a high-pressure technique, e.g., POWDERJECT®. Surgical techniques include implantation of depot (reservoir) compositions, osmotic pumps, and the like. A preferred route of administration for treatment of inflammation can be local or topical delivery for localized disorders such as arthritis, or systemic delivery for distributed disorders, e.g., intravenous delivery for reperfusion injury or for systemic conditions such as septicemia. For other diseases, including those involving the respiratory tract, e.g., chronic obstructive pulmonary disease, asthma, emphysema, etc., administration may be accomplished by inhalation or deep lung administration of sprays, aerosols, powders, and the like.

For the treatment of neoplastic diseases, especially leukemias and other distributed cancers, parenteral administration is typically preferred. Formulations of the compounds to optimize them for biodistribution following parenteral administration would be desirable. The PI3Kδ inhibitor compounds may be administered before, during, or after administration of chemotherapy, radiotherapy, and/or surgery.

Moreover, the therapeutic index of the p110δ:LASP-1 modulator compounds may be enhanced by modifying or derivatizing the compounds for targeted delivery to cancer cells expressing a marker that identifies the cells as such. For example, the compounds may be linked to an antibody that recognizes a marker that is selective or specific for cancer cells, so that the compounds are brought into the vicinity of the cells to exert their effects locally, as previously described [see for example, Pietersz et al., *Immunol Rev* 129:57 (1992); Trail et al., *Science* 261:212 (1993); and Rowlinson-Busza et al., *Curr Opin Oncol* 4:1142 (1992)]. Tumor-directed delivery of these compounds would enhance the therapeutic benefit by, inter alia, minimizing potential non-specific toxicities that can result from radiation treatment or chemotherapy. In another aspect, p110δ:LASP-1 inhibitor compounds and radioisotopes or chemotherapeutic agents may be conjugated to the same anti-tumor antibody.

For the treatment of bone resorption disorders or osteoclast-mediated disorders, the p110δ:LASP-1 modulators can be delivered by any suitable method. Focal administration may be desirable, such as by intraarticular injection. In some cases, it may be desirable to couple the compounds to a moiety that can target the compounds to bone. For example, a p110δ:LASP-1 modulator may be coupled to compounds with high affinity for hydroxyapatite, which is a major constituent of bone. This may be accomplished, for example, by adapting a tetracycline-coupling method developed for targeted delivery of estrogen to bone [Orme et al., *Bioorg Med Chem Lett* 4(11):1375-80 (1994)].

To be effective therapeutically in modulating central nervous system targets, the agents used in the methods of the invention should readily penetrate the blood brain barrier when peripherally administered. Compounds that cannot penetrate the blood brain barrier, however, can still be effectively administered by an intravenous route.

As noted above, the characteristics of the agent itself and the formulation of the agent can influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the administered agent. Such pharmacokinetic and pharmacodynamic information can be collected through pre-clinical *in vitro* and *in vivo* studies, later confirmed in humans during the course of clinical trials. Thus, for

any compound used in the method of the invention, a therapeutically effective dose can be estimated initially from biochemical and/or cell-based assays. Then, dosage can be formulated in animal models to achieve a desirable circulating concentration range that modulates p110δ:LASP-1 binding. As human studies are conducted, further information will emerge regarding the appropriate dosage levels and duration of treatment for various diseases and conditions.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the "therapeutic index," which is typically expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit large therapeutic indices are preferred. The data obtained from such cell culture assays and additional animal studies can be used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity.

For the method of the invention, any effective administration regimen regulating the timing and sequence of doses may be used. Doses of the agent preferably include pharmaceutical dosage units comprising an effective amount of the agent. As used herein, "effective amount" refers to an amount of a p110δ:LASP-1 modulator compound sufficient to modulate PI3Kδ activity and/or derive a measurable change in a physiological parameter of the subject through administration of one or more of the pharmaceutical dosage units.

Exemplary dosage levels for a human subject are of the order of from about 0.001 milligram of active agent per kilogram body weight (mg/kg) to about 100 mg/kg. Typically, dosage units of the active agent comprise from about 0.01 mg to about 10,000 mg, preferably from about 0.1 mg to about 1,000 mg, depending upon the indication, route of administration, etc. Depending on the route of administration, a suitable dose may be calculated according to body weight, body surface area, or organ size. The final dosage regimen will be determined by the attending physician in view of good medical practice, considering various factors that modify the action of drugs, e.g., the agent's specific activity, the severity of the disease state, the

responsiveness of the patient, the age, condition, body weight, sex, and diet of the patient, the severity of any infection, etc. Additional factors that may be taken into account include time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Further refinement of the dosage appropriate for treatment involving any of the formulations mentioned herein is done routinely by the skilled practitioner without undue experimentation, especially in light of the dosage information and assays disclosed, as well as the pharmacokinetic data observed in human clinical trials. Appropriate dosages may be ascertained through use of established assays for determining concentration of the agent in a body fluid or other sample together with dose response data.

The frequency of dosing will depend on the pharmacokinetic parameters of the agent and the route of administration. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Accordingly, the pharmaceutical compositions can be administered in a single dose, multiple discrete doses, continuous infusion, sustained release depots, or combinations thereof, as required to maintain desired minimum level of the agent. Short-acting pharmaceutical compositions (i.e., short half-life) can be administered once a day or more than once a day (e.g., two, three, or four times a day). Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks. Pumps, such as subcutaneous, intraperitoneal, or subdural pumps, may be preferred for continuous infusion.

The following Examples are provided to further aid in understanding the invention, and presuppose an understanding of conventional methods well-known to those persons having ordinary skill in the art to which the examples pertain, e.g., the construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids, or the introduction of vectors and plasmids into host cells. Such methods are described in detail in numerous publications including, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989), Ausubel et al. (Eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994); and Ausubel et al. (Eds.), *Short Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc. (1999). The particular materials and conditions described hereunder are intended to exemplify

particular aspects of the invention and should not be construed to limit the reasonable scope thereof.

EXAMPLE 1. Identification and Verification of a p110δ-Interacting Partner

The yeast di-hybrid technique was used in an effort to identify proteins that interact with the 110 kDa subunit of PI3 kinase delta (p110δ). The di-hybrid method is described by Fields et al. [*Nature* 340(6230):245-6 (1989)]. [See also, e.g., U.S. Patent No. 5,959,079; Hollenberg et al., *Mol Cell Biol* 15:3813-22 (1995)].

Construction of Bait Plasmid and Yeast Strains

As shown in Table 1, a comparison of the amino acid sequence of human p110δ to those of the other three p110 subunit types reveals that the amino acid sequence of the Ras-binding domain is a less conserved region than the catalytic domain.

Table 1

PI3K Isoform	Identity		
	Overall	**Catalytic**	**Ras-Binding**
p110α (SEQ ID NO:6)	41	49	26
p110β (SEQ ID NO:8)	58	72	35.5
p110γ (SEQ ID NO:10)	35	45	20

To increase the probability of identifying proteins that specifically interact with p110δ, we took advantage of the low level of homology in the Ras-binding domains. The Ras-binding region of p110δ (aa 134 to 350 of SEQ ID NO:2) was amplified by PCR using 100 ng of template p110δ DNA (SEQ ID NO:1) and 500 ng each of the following primers.

p110delta 5': 5'-GATCGAATTCCCAGAAGTGAACGACTTTCGC-3'

(SEQ ID NO:11)

p110delta 3': 5'-GATCGTCGACGCCGTGGAAAAGCCCGGCCTG-3'

(SEQ ID NO:12)

The amplification reaction was performed using Taq polymerase and buffer provided by the supplier (Perkin Elmer, Foster City CA) according to the following temperature cycling protocol: 94°C for 5 min; followed by 25 cycles of 94°C for 30 sec, 68°C for 3 min, and 72° for 7 min. The amplified product was then digested with *Eco*R1 and *Sal*1 and subcloned in the *Eco*R1-*Sal*1 sites of the yeast di-hybrid vector pBTM116 such that the p110δ sequence was in frame with the LexA sequence.

We also cloned the corresponding region of p110β (aa 143 to 360) in frame with the LexA sequence for use as a specificity control. The following oligonucleotides were used to amplify the Ras-binding domain of p110β sequence (SEQ ID NO:8).

p110beta 5 : 5 -GATCGAATTCCCTGAAGTAAATGAATTTCGA-3

(SEQ ID NO:13)

p110beta 3 : 5 -GATCGTCGACACCATGAAAAAGACCAGCCCT-3

(SEQ ID NO:14)

The pBTM116 plasmid DNAs containing the p110δ or p110β sequences were then introduced into yeast cells by the lithium acetate treatment, generally in accordance with the method of Ito et al. [*J Bacteriol* 153:163-8 (1983)].

Screening for p110δ-Interacting Clones

One yeast colony containing the p110δ Ras-binding sequence was grown and the cells were transformed with a mouse embryonic cDNA library in the yeast vector pVP16. In this vector, the mouse cDNAs are cloned in frame with the VP16 sequence so that the resulting fusion protein can recognize RNA polymerase using the VP16 sequence to help initiate transcription. The cells were plated on appropriate selection medium lacking leucine, tryptophan and histidine. The ability of the yeast transformants to survive and propagate on this medium suggests an interaction

between p110δ and the protein coded by the mouse cDNA sequence. As a further test of protein:protein interaction, replicas of the yeast colonies on the selection plates were made on membranes and assayed for β-galactosidase activity by a standard method [Breeden et al. *Cold Spring Harbor Symp Quant Biol 50:643-50 (1985)]*. Several hundred blue colonies were observed. The colonies that did not turn blue were considered false positive and discarded.

We selected 40 of the colonies that turned blue most rapidly and isolated plasmid DNA from each of them. *E. coli* cells that require leucine for growth were transformed with each of these 40 plasmid DNAs and plated on medium lacking leucine so that the cells carrying VP16 based plasmids would be selected. Plasmid DNAs were isolated from the *E. coli* transformants and re-introduced into the yeast cells carrying pBTM116-p110δ Ras-binding domain. The resulting yeast transformants were again tested for the interaction with p110δ by growth on medium lacking leucine, tryptophan, and histidine, and also assayed for β-galactosidase activity. Only those colonies that reproducibly tested positive for the interaction were selected for further characterization.

Characterization of p110δ-Interacting Clones

Sequence Analysis

We determined the DNA sequences of 39 clones that were reproducibly positive for interaction with the p110δ region of interest (aa 134 to 350 of SEQ ID NO:2). All of the DNA sequences were used to search the GenBank® database for any related proteins. Our searches revealed that the DNA sequence of Clone 32 (SEQ ID NO:15) is highly homologous with portions of the human and mouse LASP-1 DNAs (GenBank® Accession Nos. NM_006148 (human; SEQ ID NO:3) and NM_010688 (mouse; SEQ ID NO:17). The LASP-1 proteins of rabbit, human and mouse contain 2 domains: one LIM motif and one domain of Src homology region 3 (SH3), hence its name "LIM and SH3 Protein" [Tomasetto et al., *FEBS Lett* 373:245-9 (1995); Chew et al., *Amer J Physiol* 275 (*Cell Physiol* 44):C56-C57 (1998)]. The amino acid sequence of the SH3 domain of Clone 32 (aa 4-60 of SEQ ID NO:16) was identical to the SH3 domain of both the human and mouse LASP-1 proteins (aa 205-261 of SEQ ID NO:4 and aa 207-263 of SEQ ID NO:18, respectively), which are

identical to one another. Moreover, the amino acid sequences of the Ras-binding domains of mouse and human p110 are 95% conserved. In particular, the amino acid sequences of the proline-rich segments within the Ras-binding domains of both the human and mouse p110δ protein are identical. Since the mouse and human LASP-1 SH3 domain amino acid sequences are identical, and since the mouse and human p110δ proline-rich segments are identical, we surmise that human LASP-1 is capable of binding to the human p110δ proline-rich segment as does clone 32 which is derived from mouse LASP-1.

The DNA sequence of the Clone 32 is given below (note that the *Not*I sites (underlined) at the 5' and 3' ends are from the vector).

GCGGCCGCTCAAACGGTACCGTGCAGTGTATGACTACAGCGCTGCCGACGAGGACGA
GGTCTCCTTCCAGGATGGGGACACCATCGTCAATGTGCAGCAGATCGATGACGGCTG
GATGTACGGGACCGTAGAGCGCACCGGTGACACGGGGATGCTGCCAGCCAACTACGT
GGAGGCCATCTGAACCCTGTGCCGCCCCGCCCTGTCTTCAATGCATTCCATGGCATC
ACATCTGTCCTGGGGCCTGACCCGTCCACCCTACAGTGTCTCTGTCTTTTAAGATCT
TCAACTGCTTCTTTATCCCCGCCCCTCCAGCTTATTTTACCATCCCAAGCCTTGTTC
TGCCCCCTGAGCGGCCGC

(SEQ ID NO:15)

Since the mouse cDNAs are fused to the coding sequence of VP16, we deduced that the polypeptide sequence encoded by Clone 32 (below) was in the same reading frame as VP16. As noted above, amino acids 4-60 of SEQ ID NO:16 correspond to the amino terminal region of LASP-1.

RPLKRYRAVYDYSAADEDEVSFQDGDTIVNVQQIDDGWMYGTVERTGDTGMLPANYV
EAI

(SEQ ID NO:16)

Test for Specificity of Interaction of p110δ with Clone 32

To check for the specificity of interaction of Clone 32 with the PI3 kinases, we introduced the Clone 32 plasmid DNA into the yeast cells carrying the p110β Ras-binding domain, since the p110β sequence is closest to the p110δ sequence. After selection on appropriate growth medium, the yeast transformants were assayed for β-galactosidase activity. The cells carrying the p110β sequence did not show any β-galactosidase activity whereas the p110δ-carrying cells again showed positive activity, suggesting an absence of interaction between Clone 32 and p110β Ras-binding domain (Table 2).

Clone 32 Interacts with the Proline (Pro) Rich Region of p110δ

As determined by sequence analysis, Clone 32 comprises an SH3 domain. Since SH3 domains are known to bind proline rich sequences, we tested whether Clone 32 binds to the proline rich sequence of p110δ. We created 3 individual clones, each carrying one of the following mutations: 1) P304/A, 2) P307/A, and 3) deletion of 51 amino acids (aa 300-350 of SEQ ID NO:2) into our original pBTM116-p110δ Ras-binding domain construct using standard recombinant DNA techniques. Sequences of the proline-rich segments of p110β and p110δ are given below. In the p110δ sequence, the proline residues mutated to provide the P304/A and P307/A clones are identified.

p110δ: EQSNPAPQVQKPRAK----PPPIPAKKPSSV

(aa 288-314 of SEQ ID NO:2)

p110β: EQEMIAIEAAINRNSSNLPLPLPPKKTRIIS

(aa 294-324 of SEQ ID NO:8)

The three mutant plasmids were introduced into yeast cells and tested for interaction with Clone 32 by growth on selective medium and β-galactosidase activity. Growth of the cells containing Clone 32 or mutant plasmids were poor. Moreover, as shown in Table 2, change of any of the proline residues to alanine

resulted in a reduction of β-galactosidase activity. Deletion of the proline rich segment eliminated β-galactosidase activity. These results suggest that the Clone 32 (which encodes an SH3 domain) product interacts with p110δ (aa 134-350 of SEQ ID NO:2) through the proline rich segment of p110δ.

Table 2

Bait	β-Galactosidase Activity
p110β	-
p110δ	++
p110δ (P304/A)	-
p110δ (P307/A)	+/-
p110δ (Pro Rich Segment Deletion)	-

Further Verification of Interaction of p110δ Ras-Binding Domain with Clone 32

Since the yeast di-hybrid technique can produce protein:protein interactions that are not always reproducible in mammalian cells, we tested whether Clone 32 can interact with p110δ within a mammalian cell environment. We generated a fusion of GFP (Green Florescent Protein) with Clone 32 in the vector pcDNA3.1/NT-GFP-TOPO (Invitrogen, San Diego, CA) according to the procedure recommended by the supplier. The resulting fusion plasmid, designated "GFP-32," was co-transfected in triplicate with a FLAG-p110δ-containing plasmid [Chantry et al., *J Biol Chem* 272:19236-41 (1997)] into the human embryonic kidney cell line HEK293. (The FLAG® N-terminal peptide identification system is described e.g., in US Patent Nos. 4,851,341 and 5,011,912.) Forty-eight hours after transfection the cells were lysed and immunoprecipitated using anti-FLAG® antibody M2-coated agarose beads. The bound proteins were separated by SDS-PAGE, blotted on nylon membrane, and probed with a rabbit anti-GFP antibody (Clontech, Palo Alto CA) and rabbit anti-p110δ antibodies (prepared using the method described in US Patent No. 5,882,910). A horseradish peroxidase (HRP)-linked goat anti-rabbit antibody (Clontech) was used according to the manufacturerÅs protocol to develop the blot.

Examination of the developed blot showed a band at about 33 kDa in the lanes containing lysates of cells transfected with the plasmid pcDNA3.1/NT-GFP, indicating expression of the GFP protein only. In the lanes in which lysates from FLAG-p110δ and pcDNA3.1/NT-GFP-clone32 co-transfected cells were applied, both the FLAG-p110δ band (at about 110 kDa) and the GFP-clone32 band (at about 40 kDa) were observed, indicating expression of both proteins.

We also analyzed the supernatant and pellet of anti-FLAG® immunoprecipitated samples for the presence of FLAG-p110δ and GFP-clone32. As expected, the supernatants contained very little FLAG-p110δ signal, indicating successful precipitation of FLAG-p110δ. Complementing the absence of FLAG-p110δ in the supernatant, there was a large increase in the FLAG-p110δ signal in the pellets. In addition to the FLAG-p110δ signal, another band was observed in the pellet. This band comigrated with the GFP-clone32 band. These results indicate that Clone 32 can associate with p110δ within the mammalian cellular environment.

LASP-1 has been shown to be phosphorylated in response to extracellular signals such as forskolin, and the phosphorylation is correlated to the secretory response of the cells [Chew et al., *Am J Physiol* 275 (*Cell Physiol* 44:C56-C57) (1998)]. In addition, LASP-1 has been shown to be tyrosine phosphorylated by c-Src and binds to actin [Schreiber et al., *Mol Med* 4:675-687 (1998)]. Since p110δ is involved in cellular signal transduction pathways, we surmise that the LASP-1: p110δ interaction may facilitate secretion and/or cytoskeletal reorganization. Disruption of the interaction between p110δ proline rich sequence and SH3 domains may prevent p110δ activation in response to specific stimulus in a given cell type and thereby prevent unwanted signaling through the p110δ pathway. Disease states mediated by excessive or undesirable p110δ activity, therefore, may be expected to be susceptible to treatment using modulator compounds that disrupt binding or interaction of p110δ with LASP-1.

EXAMPLE 2. Functional Significance of the Proline-rich Sequence in p110δ

SH3 domains are approximately sixty amino acid residues long and are found in many signaling proteins, enzymes, and cytoskeletal proteins. Despite their amino

acid sequence diversity, all SH3 domains bind to a short stretch of polyproline sequences of 8 to 10 amino acid residues. Even though several hundred distinct SH domains are known in human, they seem to bind polyproline sequences with a remarkable degree of specificity. The interaction between a polyproline sequence and a SH3 domain may be intramolecular or intermolecular. In the former case, the polyproline sequence and the SH3 domain of the same polypeptide chain bind to each other. In case of intermolecular, interactions the polyproline sequence and the SH3 domain from different proteins interact.

Polyproline sequences serve as ligands for many protein domains such as the EVH1 (Enabled, VASP Homolgy 1), WW and SH3 (Src Homology 3) for example. These protein domains and their ligand polyproline sequences have been observed in many species suggesting their evolutionary conserved role in cellular functions. Among the different domains that bind polyproline sequences, the SH3 domain has been studied most of all.

SH3 binding has been shown to activate signaling enzymes as well as in the formation of active signaling complexes [Pleiman et al., *Science* 263:1609-1612 (1994); Pawson, *Nature* 373:573-580 (1995)]. The present study shows that the proline-rich segment of p110δ can function as an SH3 binding sequence. Hence, it is possible that binding of SH3 domain-containing proteins leads to the activation of the kinase activity of p110δ. For example, Src family kinases and adapter proteins (e.g., vav, cbl) having one or more SH3 domains may bind to p110δ through the proline-rich domain.

Due to the important roles of polyproline sequences in the regulation of cellular activation, small molecule inhibitors are being developed to prevent the interaction between polyproline sequences and their cognate binding partners. Proline is the only amino acid where the amido N is substituted. Based on this unique feature of proline, Nguyen et al., *Science*, 282: 2088-2092 (1998) designed a set of N-substituted analogs that can inhibit SH3-polyproline interaction.

Indications for p110δ Activity Requirement in Neutrophils

Treatment of neutrophils with a p110δ-specific inhibitor (such as disclosed in U.S.S.N. 09/841,341 filed April 24, 2001, the disclosure of which is hereby

incorporated by reference) results in the inhibition of neutrophil functions such as superoxide production. NADPH oxidase, the enzyme responsible for superoxide production, is a multicomponent enzyme, and p47-phox is an essential constituent of the complex. P47-phox is described in Volpp et.al., *PNAS*, 86: 7195-7199 (1989). The p47-phox protein contains two SH3 domains. Human mutations of p47-phox have been discovered that result in defects in superoxide production by neutrophils. Most of these p47-phox mutations cause premature truncation and as a result the SH3 domains are not synthesized. These observations establish that in addition to p110δ, p47-phox is also necessary for superoxide production. Since, both p110δ and p47-phox are required, it is contemplated that the polyproline sequence of p110δ functions in superoxide synthesis through an interaction with SH3 domain of p47-phox.

Indication for p110δ Activity Requirement in B cells

In addition to inhibiting neutrophil function, p110δ-specific inhibitor blocks B cell proliferation. This suggests that p110δ plays an important role in B cell function. Bruton's tyrosine kinase (Btk) is another kinase that plays a crucial role in B cell function. Btk is described in Vetri et al., *Nature*, 361: 226-233 (1993) and Tsukata et al., *Cell*, 72: 279-290 (1993). Btk is preferentially expressed in B cells, mast cells and platelets. Btk is an X-linked gene, and defects in Btk activity results in X-linked agammaglobulinemia (XLA). Primary symptoms of XLA are low numbers of peripheral B cells and cosequently low antibody titer. Similar to the defects in human B cells, in mice loss of Btk results in defective B cell development and function. A remarkably similar defect in B cell compartment was observed in PI kinase subunit p85 knock out mice. Since p85 is an essential component of class IA PI3 kinases, in addition to Btk, PI3 kinase(s) are apparently required for B cell function. Since Btk has an SH3 domain, it is contemplated that p110δ interacts with the SH3 domain of Btk through its polyproline sequence.

In neutrophils and B cells therefore, it is contemplated that the polyproline sequence of p110δ is involved in interactions with the SH3 domains of p47-phox and Btk respectively. However, in addition to interacting with other proteins, the polyproline sequence of p110δ can interact with components of PI3 kinase itself. It may be recalled that class IA kinases consist of a catalytic subunit (p10) and a

regulatory subunit (p85). P85 is described in Escobedo et al., *Cell*, 65: 75-82 (1991); Skolnik et al., *Cell,* 65: 83-90 (1991) and Otsu et al., *Cell*, 65: 91-104 (1991). Association of p85 is essential for the catalytic activity of p110. Though the regions responsible for the binding of p85 subunit to p110 subunit have been mapped, it is contemplated that the p110 subunit of PI3 kinase delta provides an additional binding site to the SH3 domain of p85 through its unique polyproline region, thereby further enhancing the interaction between p85 and p110δ.

In summary the above example illustrates binding of the proline-rich sequence of p110δ to SH3 domains of other proteins (e.g., p47-phox, Btk) or a component of PI3 kinase itself (e.g., p85) and regulation of cellular activity.

All publications and patent documents cited in this specification are incorporated herein by reference for all that they disclose.

While the present invention has been described with specific reference to certain preferred embodiments for purposes of clarity and understanding, it will be apparent to the skilled artisan that further changes and modifications may be practiced within the scope of the invention as it is defined in the claims set forth below. Accordingly, no limitations should be placed on the invention other than those specifically recited in the claims.

WHAT IS CLAIMED IS:

1. A method of identifying a modulator of p110δ binding to LASP-1, comprising the steps of:

(a) providing a p110δ polypeptide and a LASP-1 polypeptide having specific binding affinity for one another;

(b) measuring binding between the p110δ polypeptide and the LASP-1 polypeptide in the presence and absence of a test compound; and

(c) identifying the test compound as a modulator of p110δ binding to LASP-1 when a different amount of binding of the p110δ polypeptide to the LASP-1 polypeptide is detected in the presence of the test compound than in the absence of the test compound.

2. A method according to Claim 1, wherein the p110δ polypeptide comprises the proline rich region of p110δ.

3. A method according to Claim 1, wherein the LASP-1 polypeptide comprises the SH3 region of LASP-1.

4. A method according to Claim 1, wherein the p110δ polypeptide is provided as a fusion protein.

5. A method according to Claim 1, wherein the LASP-1 polypeptide is provided as a fusion protein.

6. A method according to Claim1, wherein the p110δ polypeptide is expressed by a host cell.

7. A method according to Claim 1, wherein the LASP-1 polypeptide is expressed by a host cell.

8. A method according to Claim 1, wherein the p110δ polypeptide and the LASP-1 polypeptide are recombinantly coexpressed by a host cell.

9. A method according to Claim 1, wherein the modulator inhibits binding of p110δ to LASP-1.

10. A method according to Claim 1, wherein the modulator enhances binding of p110δ to LASP-1.

11. A compound having activity as a modulator of interaction between p110δ and LASP-1, wherein the compound is identified according to the method of Claim 1.

12. A compound according to Claim 11, wherein the compound inhibits binding of p110δ to LASP-1.

13. A method of treating a disease state characterized by undesirable or excessive activity of PI3Kδ, comprising administering to a subject in need thereof a compound according to Claim 11 or a derived analog thereof in an amount effective to inhibit interaction of p110δ with LASP-1.

14. A method according to Claim 13, wherein the modulator inhibits binding of p110δ to LASP-1.

15. A method of modulating p110δ binding to LASP-1, comprising the step of contacting p110δ or LASP-1 with a modulator of p110δ binding to LASP-1.

16. A method according to Claim 15, wherein the modulator inhibits binding of p110δ to LASP-1.

17. A method of treating a disease state associated with p110δ binding to LASP-1, comprising the step of administering to a subject in need thereof an effective amount of a modulator of p110δ binding to LASP-1.

18. A method according to Claim 17, wherein the modulator inhibits binding of p110δ to LASP-1.

19. A method of identifying a modulator of p110δ binding to a polypeptide comprising an SH3 domain, comprising the steps of:

(a) providing a p110δ polypeptide and an SH3 domain-containing polypeptide having specific binding affinity for one another;

(b) measuring binding between the p110δ polypeptide and the SH3 domain-containing polypeptide in the presence and absence of a test compound; and

(c) identifying the test compound as a modulator of p110δ binding to the SH3 domain-containing polypeptide when a different amount of binding of the p110δ polypeptide to the SH3 domain-containing polypeptide is detected in the presence of the test compound than in the absence of the test compound.

20. A method according to Claim 19, wherein the p110δ polypeptide is provided as a fusion protein.

21. A method according to Claim 19, wherein the SH3 domain-containing polypeptide is provided as a fusion protein.

22. A method according to Claim19, wherein the p110δ polypeptide is expressed by a host cell.

23. A method according to Claim 19, wherein the SH3 domain-containing polypeptide is expressed by a host cell.

24. A method according to Claim 19, wherein the p110δ polypeptide and the SH3 domain-containing polypeptide are recombinantly coexpressed by a host cell.

25. A method according to Claim 19, wherein the modulator inhibits binding of p110δ to the SH3 domain-containing polypeptide.

26. A method according to Claim 19, wherein the modulator enhances binding of p110δ to the SH3 domain-containing polypeptide.

27. A compound having activity as a modulator of interaction between p110δ and an SH3 domain-containing polypeptide, wherein the compound is identified according to the method of Claim 19.

28. A compound according to Claim 28, wherein the compound inhibits binding of p110δ to the SH3 domain-containing polypeptide.

29. A method of treating a disease state characterized by undesirable or excessive activity of PI3Kδ, comprising administering to a subject in need thereof a compound according to Claim 27.

30. A method according to Claim 29, wherein the compound inhibits binding of p110δ to the SH3 domain-containing polypeptide.

31. A method of modulating p110δ binding to an SH3 domain-containing polypeptide, comprising the step of contacting p110δ or the SH3 domain-containing polypeptide with a modulator of p110δ binding to the SH3 domain-containing polypeptide.

32. A method according to Claim 31, wherein the modulator inhibits binding of p110δ to the SH3 domain-containing polypeptide.

33. A method of treating a disease state associated with p110δ binding to an SH3 domain-containing polypeptide, comprising the step of administering to a subject in need thereof an effective amount of a modulator of p110δ binding to the SH3 domain-containing polypeptide.

34. A method according to Claim 33, wherein the modulator inhibits binding of p110δ to the SH3 domain-containing polypeptide.

35. The method of claim 19, 29, 31 or 33 wherein the SH3 domain-containing polypeptide is p85.

36. The method of claim 19, 29, 31 or 33 wherein the SH3 domain-containing polypeptide is p47-phox.

37. The method of claim 19, 29, 31 or 33 wherein the SH3 domain-containing polypeptide is Btk.

SEQUENCE LISTING

<110> Sadhu, Chanchal

<120> PHOSPHATIDYL INOSITOL 3-KINASE DELTA BINDING PARTNER

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445                 450                 455                 460

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Ser Asn Pro Asn Thr Asp Ser Ala Ala Ala Leu Leu Ile Cys Leu Pro
                465                 470                 475

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            480                 485                 490

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            960                 965                 970

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Leu Phe Leu His Leu Phe Ala Leu Met Arg Ala Ala Gly Leu Pro Glu
        975                 980                 985

ctc agc tgc tcc aaa gac atc cag tat ctc aag gac tcc ctg gca ctg   3208
Leu Ser Cys Ser Lys Asp Ile Gln Tyr Leu Lys Asp Ser Leu Ala Leu
    990                 995                1000

ggg aaa aca gag gag gag gca ctg aag cac ttc cga gtg aag ttt aac   3256
Gly Lys Thr Glu Glu Glu Ala Leu Lys His Phe Arg Val Lys Phe Asn
1005                1010                1015                1020

gaa gcc ctc cgt gag agc tgg aaa acc aaa gtg aac tgg ctg gcc cac   3304
Glu Ala Leu Arg Glu Ser Trp Lys Thr Lys Val Asn Trp Leu Ala His
                1025                1030                1035

aac gtg tcc aaa gac aac agg cag tag tggctcctcc cagccctggg         3351
Asn Val Ser Lys Asp Asn Arg Gln
            1040                1045

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gagcctgaac tgcacctaac gggaaagaac cgacatggct gccttttgtt tacactggtt 3471

atttatttat gacttgaaat agtttaagga gctaaacagc cataaacgga aacgcctcct 3531
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35 40 45

Leu Trp His Arg Ala Gln Tyr Glu Pro Leu Phe His Met Leu Ser Gly
50 55 60

Pro Glu Ala Tyr Val Phe Thr Cys Ile Asn Gln Thr Ala Glu Gln Gln
65 70 75 80

Glu Leu Glu Asp Glu Gln Arg Arg Leu Cys Asp Val Gln Pro Phe Leu
85 90 95

Pro Val Leu Arg Leu Val Ala Arg Glu Gly Asp Arg Val Lys Lys Leu
100 105 110

Ile Asn Ser Gln Ile Ser Leu Leu Ile Gly Lys Gly Leu His Glu Phe
115 120 125

Asp Ser Leu Cys Asp Pro Glu Val Asn Asp Phe Arg Ala Lys Met Cys
130 135 140

Gln Phe Cys Glu Glu Ala Ala Ala Arg Arg Gln Gln Leu Gly Trp Glu
145 150 155 160

Ala Trp Leu Gln Tyr Ser Phe Pro Leu Gln Leu Glu Pro Ser Ala Gln
165 170 175

Thr Trp Gly Pro Gly Thr Leu Arg Leu Pro Asn Arg Ala Leu Leu Val
180 185 190

Asn Val Lys Phe Glu Gly Ser Glu Glu Ser Phe Thr Phe Gln Val Ser
195 200 205

Thr Lys Asp Val Pro Leu Ala Leu Met Ala Cys Ala Leu Arg Lys Lys
210 215 220

Ala Thr Val Phe Arg Gln Pro Leu Val Glu Gln Pro Glu Asp Tyr Thr
225 230 235 240

Leu Gln Val Asn Gly Arg His Glu Tyr Leu Tyr Gly Ser Tyr Pro Leu
245 250 255

Cys Gln Phe Gln Tyr Ile Cys Ser Cys Leu His Ser Gly Leu Thr Pro
260 265 270

His Leu Thr Met Val His Ser Ser Ser Ile Leu Ala Met Arg Asp Glu
275 280 285

Gln Ser Asn Pro Ala Pro Gln Val Gln Lys Pro Arg Ala Lys Pro Pro
290 295 300

Pro Ile Pro Ala Lys Lys Pro Ser Ser Val Ser Leu Trp Ser Leu Glu
305 310 315 320

Gln Pro Phe Arg Ile Glu Leu Ile Gln Gly Ser Lys Val Asn Ala Asp
325 330 335

Glu Arg Met Lys Leu Val Val Gln Ala Gly Leu Phe His Gly Asn Glu
340 345 350

Met Leu Cys Lys Thr Val Ser Ser Ser Glu Val Ser Val Cys Ser Glu
355 360 365

Pro Val Trp Lys Gln Arg Leu Glu Phe Asp Ile Asn Ile Cys Asp Leu
370 375 380

Pro Arg Met Ala Arg Leu Cys Phe Ala Leu Tyr Ala Val Ile Glu Lys
385 390 395 400

Ala Lys Lys Ala Arg Ser Thr Lys Lys Lys Ser Lys Lys Ala Asp Cys
405 410 415

Pro Ile Ala Trp Ala Asn Leu Met Leu Phe Asp Tyr Lys Asp Gln Leu
420 425 430

Lys Thr Gly Glu Arg Cys Leu Tyr Met Trp Pro Ser Val Pro Asp Glu
435 440 445

Lys Gly Glu Leu Leu Asn Pro Thr Gly Thr Val Arg Ser Asn Pro Asn
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Thr Asp Ser Ala Ala Ala Leu Leu Ile Cys Leu Pro Glu Val Ala Pro
465 470 475 480

His Pro Val Tyr Tyr Pro Ala Leu Glu Lys Ile Leu Glu Leu Gly Arg
485 490 495

His Ser Glu Cys Val His Val Thr Glu Glu Glu Gln Leu Gln Leu Arg
500 505 510

Glu Ile Leu Glu Arg Arg Gly Ser Gly Glu Leu Tyr Glu His Glu Lys
515 520 525

Asp Leu Val Trp Lys Leu Arg His Glu Val Gln Glu His Phe Pro Glu
530 535 540

Ala Leu Ala Arg Leu Leu Leu Val Thr Lys Trp Asn Lys His Glu Asp
545 550 555 560

Val Ala Gln Met Leu Tyr Leu Leu Cys Ser Trp Pro Glu Leu Pro Val
565 570 575

Leu Ser Ala Leu Glu Leu Leu Asp Phe Ser Phe Pro Asp Cys His Val
580 585 590

Gly Ser Phe Ala Ile Lys Ser Leu Arg Lys Leu Thr Asp Asp Glu Leu
595 600 605

Phe Gln Tyr Leu Leu Gln Leu Val Gln Val Leu Lys Tyr Glu Ser Tyr
610 615 620

Leu Asp Cys Glu Leu Thr Lys Phe Leu Leu Asp Arg Ala Leu Ala Asn
625 630 635 640

Arg Lys Ile Gly His Phe Leu Phe Trp His Leu Arg Ser Glu Met His
645 650 655

Val Pro Ser Val Ala Leu Arg Phe Gly Leu Ile Leu Glu Ala Tyr Cys
660 665 670

Arg Gly Arg Thr His His Met Lys Val Leu Met Lys Gln Gly Glu Ala
675 680 685

Leu Ser Lys Leu Lys Ala Leu Asn Asp Phe Val Lys Leu Ser Ser Gln
690 695 700

Lys Thr Pro Lys Pro Gln Thr Lys Glu Leu Met His Leu Cys Met Arg
705 710 715 720

Gln Glu Ala Tyr Leu Glu Ala Leu Ser His Leu Gln Ser Pro Leu Asp
725 730 735

Pro Ser Thr Leu Leu Ala Glu Val Cys Val Glu Gln Cys Thr Phe Met
740 745 750

Asp Ser Lys Met Lys Pro Leu Trp Ile Met Tyr Ser Asn Glu Glu Ala
755 760 765

Gly Ser Gly Gly Ser Val Gly Ile Ile Phe Lys Asn Gly Asp Asp Leu
770 775 780

Arg Gln Asp Met Leu Thr Leu Gln Met Ile Gln Leu Met Asp Val Leu
785 790 795 800

Trp Lys Gln Glu Gly Leu Asp Leu Arg Met Thr Pro Tyr Gly Cys Leu
805 810 815

Pro Thr Gly Asp Arg Thr Gly Leu Ile Glu Val Val Leu Arg Ser Asp
820 825 830

Thr Ile Ala Asn Ile Gln Leu Asn Lys Ser Asn Met Ala Ala Thr Ala
835 840 845

Ala Phe Asn Lys Asp Ala Leu Leu Asn Trp Leu Lys Ser Lys Asn Pro
850 855 860

Gly Glu Ala Leu Asp Arg Ala Ile Glu Glu Phe Thr Leu Ser Cys Ala
865 870 875 880

Gly Tyr Cys Val Ala Thr Tyr Val Leu Gly Ile Gly Asp Arg His Ser
885 890 895

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Asp Asn Ile Met Ile Arg Glu Ser Gly Gln Leu Phe His Ile Asp Phe
            900                 905                 910

Gly His Phe Leu Gly Asn Phe Lys Thr Lys Phe Gly Ile Asn Arg Glu
        915                 920                 925

Arg Val Pro Phe Ile Leu Thr Tyr Asp Phe Val His Val Ile Gln Gln
    930                 935                 940

Gly Lys Thr Asn Asn Ser Glu Lys Phe Glu Arg Phe Arg Gly Tyr Cys
945                 950                 955                 960

Glu Arg Ala Tyr Thr Ile Leu Arg Arg His Gly Leu Leu Phe Leu His
                965                 970                 975

Leu Phe Ala Leu Met Arg Ala Ala Gly Leu Pro Glu Leu Ser Cys Ser
            980                 985                 990

Lys Asp Ile Gln Tyr Leu Lys Asp Ser Leu Ala Leu Gly Lys Thr Glu
        995                1000                1005

Glu Glu Ala Leu Lys His Phe Arg Val Lys Phe Asn Glu Ala Leu Arg
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                Met Asn Pro Asn Cys Ala Arg Cys Gly Lys Ile Val
                  1               5                  10

tat ccc acg gag aag gtg aac tgt ctg gat aag ttc tgg cat aaa gca  159
Tyr Pro Thr Glu Lys Val Asn Cys Leu Asp Lys Phe Trp His Lys Ala
         15                  20                  25

tgc ttc cat tgc gag acc tgc aag atg aca ctg aac atg aag aac tac  207
Cys Phe His Cys Glu Thr Cys Lys Met Thr Leu Asn Met Lys Asn Tyr
     30                  35                  40

aag ggc tac gag aag aag ccc tac tgc aac gca cac tac ccc aag cag  255
Lys Gly Tyr Glu Lys Lys Pro Tyr Cys Asn Ala His Tyr Pro Lys Gln
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tcc ttc acc atg gtg gcg gac acc ccg gaa aac ctt cgc ctc aag caa  303
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Ser Phe Thr Met Val Ala Asp Thr Pro Glu Asn Leu Arg Leu Lys Gln
                65                  70                  75

cag agt gag ctc cag agt cag gtg cgc tac aag gag gag ttt gag aag   351
Gln Ser Glu Leu Gln Ser Gln Val Arg Tyr Lys Glu Glu Phe Glu Lys
            80                  85                  90

aac aag ggc aaa ggt ttc agc gta gtg gca gac acg ccc gag ctc cag   399
Asn Lys Gly Lys Gly Phe Ser Val Val Ala Asp Thr Pro Glu Leu Gln
        95                  100                 105

aga atc aag aag acc cag gac cag atc agt aat ata aaa tac cat gag   447
Arg Ile Lys Lys Thr Gln Asp Gln Ile Ser Asn Ile Lys Tyr His Glu
    110                 115                 120

gag ttt gag aag agc cgc atg ggc cct agc ggg ggc gag ggc atg gag   495
Glu Phe Glu Lys Ser Arg Met Gly Pro Ser Gly Gly Glu Gly Met Glu
125                 130                 135                 140

cca gag cgt cgg gat tca cag gac ggc agc agc tac cgg cgg ccc ctg   543
Pro Glu Arg Arg Asp Ser Gln Asp Gly Ser Ser Tyr Arg Arg Pro Leu
                145                 150                 155

gag cag cag cag cct cac cac atc ccg acc agt gcc ccg gtt tac cag   591
Glu Gln Gln Gln Pro His His Ile Pro Thr Ser Ala Pro Val Tyr Gln
            160                 165                 170

cag ccc cag cag cag ccg gtg gcc cag tcc tat ggt ggc tac aag gag   639
Gln Pro Gln Gln Gln Pro Val Ala Gln Ser Tyr Gly Gly Tyr Lys Glu
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cct gca gcc cca gtc tcc ata cag cgc agc gcc cca ggt ggt ggc ggg   687
Pro Ala Ala Pro Val Ser Ile Gln Arg Ser Ala Pro Gly Gly Gly Gly
    190                 195                 200

aag cgg tac cgc gcg gtg tat gac tac agc gcc gcc gac gag gac gag   735
Lys Arg Tyr Arg Ala Val Tyr Asp Tyr Ser Ala Ala Asp Glu Asp Glu
205                 210                 215                 220

gtc tcc ttc cag gac ggg gac acc atc gtc aac gtg cag cag atc gac   783
Val Ser Phe Gln Asp Gly Asp Thr Ile Val Asn Val Gln Gln Ile Asp
                225                 230                 235

gac ggc tgg atg tac ggg acg gtg gag cgc acc ggc gac acg ggg atg   831
Asp Gly Trp Met Tyr Gly Thr Val Glu Arg Thr Gly Asp Thr Gly Met
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ctg ccg gcc aac tac gtg gag gcc atc tga acccggagcg cccccatctg     881
Leu Pro Ala Asn Tyr Val Glu Ala Ile
        255                 260

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Glu Thr Cys Lys Met Thr Leu Asn Met Lys Asn Tyr Lys Gly Tyr Glu
         35                  40                  45

Lys Lys Pro Tyr Cys Asn Ala His Tyr Pro Lys Gln Ser Phe Thr Met
     50                  55                  60

Val Ala Asp Thr Pro Glu Asn Leu Arg Leu Lys Gln Gln Ser Glu Leu
 65                  70                  75                  80

Gln Ser Gln Val Arg Tyr Lys Glu Glu Phe Glu Lys Asn Lys Gly Lys
                 85                  90                  95

Gly Phe Ser Val Val Ala Asp Thr Pro Glu Leu Gln Arg Ile Lys Lys
            100                 105                 110

Thr Gln Asp Gln Ile Ser Asn Ile Lys Tyr His Glu Glu Phe Glu Lys
        115                 120                 125

Ser Arg Met Gly Pro Ser Gly Gly Glu Gly Met Glu Pro Glu Arg Arg
    130                 135                 140

Asp Ser Gln Asp Gly Ser Ser Tyr Arg Arg Pro Leu Glu Gln Gln Gln
145                 150                 155                 160
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Pro His His Ile Pro Thr Ser Ala Pro Val Tyr Gln Gln Pro Gln Gln
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Gln Pro Val Ala Gln Ser Tyr Gly Gly Tyr Lys Glu Pro Ala Ala Pro
            180                 185                 190

Val Ser Ile Gln Arg Ser Ala Pro Gly Gly Gly Gly Lys Arg Tyr Arg
        195                 200                 205

Ala Val Tyr Asp Tyr Ser Ala Ala Asp Glu Asp Glu Val Ser Phe Gln
    210                 215                 220

Asp Gly Asp Thr Ile Val Asn Val Gln Gln Ile Asp Asp Gly Trp Met
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Tyr Gly Thr Val Glu Arg Thr Gly Asp Thr Gly Met Leu Pro Ala Asn
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Tyr Val Glu Ala Ile
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cac ttg atg ccc cca aga atc cta gtg gaa tgt tta cta cca aat gga   99
His Leu Met Pro Pro Arg Ile Leu Val Glu Cys Leu Leu Pro Asn Gly
     15                  20                  25

atg ata gtg act tta gaa tgc ctc cgt gag gct aca tta gta act ata   147
Met Ile Val Thr Leu Glu Cys Leu Arg Glu Ala Thr Leu Val Thr Ile
 30                  35                  40                  45

aag cat gaa cta ttt aaa gaa gca aga aaa tac cct ctc cat caa ctt   195
Lys His Glu Leu Phe Lys Glu Ala Arg Lys Tyr Pro Leu His Gln Leu
                 50                  55                  60

ctt caa gat gaa tct tct tac att ttc gta agt gtt acc caa gaa gca   243
Leu Gln Asp Glu Ser Ser Tyr Ile Phe Val Ser Val Thr Gln Glu Ala
             65                  70                  75

gaa agg gaa gaa ttt ttt gat gaa aca aga cga ctt tgt gat ctt cgg   291
Glu Arg Glu Glu Phe Phe Asp Glu Thr Arg Arg Leu Cys Asp Leu Arg
         80                  85                  90

ctt ttt caa cca ttt tta aaa gta att gaa cca gta ggc aac cgt gaa   339
Leu Phe Gln Pro Phe Leu Lys Val Ile Glu Pro Val Gly Asn Arg Glu
     95                  100                 105
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gaa aag atc ctc aat cga gaa att ggt ttt gct atc ggc atg cca gtg   387
Glu Lys Ile Leu Asn Arg Glu Ile Gly Phe Ala Ile Gly Met Pro Val
110                 115                 120                 125

tgc gaa ttt gat atg gtt aaa gat cct gaa gta cag gac ttc cga aga   435
Cys Glu Phe Asp Met Val Lys Asp Pro Glu Val Gln Asp Phe Arg Arg
                130                 135                 140

aat att ctt aat gtt tgt aaa gaa gct gtg gat ctt agg gat ctt aat   483
Asn Ile Leu Asn Val Cys Lys Glu Ala Val Asp Leu Arg Asp Leu Asn
            145                 150                 155

tca cct cat agt aga gca atg tat gtc tat ccg cca cat gta gaa tct   531
Ser Pro His Ser Arg Ala Met Tyr Val Tyr Pro Pro His Val Glu Ser
        160                 165                 170

tca cca gag ctg cca aag cac ata tat aat aaa ttg gat aga ggc caa   579
Ser Pro Glu Leu Pro Lys His Ile Tyr Asn Lys Leu Asp Arg Gly Gln
    175                 180                 185

ata ata gtg gtg att tgg gta ata gtt tct cca aat aat gac aag cag   627
Ile Ile Val Val Ile Trp Val Ile Val Ser Pro Asn Asn Asp Lys Gln
190                 195                 200                 205

aag tat act ctg aaa atc aac cat gac tgt gtg cca gaa caa gta att   675
Lys Tyr Thr Leu Lys Ile Asn His Asp Cys Val Pro Glu Gln Val Ile
                210                 215                 220

gct gaa gca atc agg aaa aaa act aga agt atg ttg cta tca tct gaa   723
Ala Glu Ala Ile Arg Lys Lys Thr Arg Ser Met Leu Leu Ser Ser Glu
            225                 230                 235

caa tta aaa ctc tgt gtt tta gaa tat cag ggc aag tac att tta aaa   771
Gln Leu Lys Leu Cys Val Leu Glu Tyr Gln Gly Lys Tyr Ile Leu Lys
        240                 245                 250

gtg tgt gga tgt gat gaa tac ttc cta gaa aaa tat cct ctg agt cag   819
Val Cys Gly Cys Asp Glu Tyr Phe Leu Glu Lys Tyr Pro Leu Ser Gln
    255                 260                 265

tat aag tat ata aga agc tgt ata atg ctt ggg agg atg ccc aat ttg   867
Tyr Lys Tyr Ile Arg Ser Cys Ile Met Leu Gly Arg Met Pro Asn Leu
270                 275                 280                 285

aag atg atg gct aaa gaa agc ctt tat tct caa ctg cca atg gac tgt   915
Lys Met Met Ala Lys Glu Ser Leu Tyr Ser Gln Leu Pro Met Asp Cys
                290                 295                 300

ttt aca atg cca tct tat tcc aga cgc att tcc aca gct aca cca tat   963
Phe Thr Met Pro Ser Tyr Ser Arg Arg Ile Ser Thr Ala Thr Pro Tyr
            305                 310                 315

atg aat gga gaa aca tct aca aaa tcc ctt tgg gtt ata aat aga gca   1011
Met Asn Gly Glu Thr Ser Thr Lys Ser Leu Trp Val Ile Asn Arg Ala
        320                 325                 330

ctc aga ata aaa att ctt tgt gca acc tac gtg aat cta aat att cga   1059
Leu Arg Ile Lys Ile Leu Cys Ala Thr Tyr Val Asn Leu Asn Ile Arg
    335                 340                 345
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gac att gac aag att tat gtt cga aca ggt atc tac cat gga gga gaa   1107
Asp Ile Asp Lys Ile Tyr Val Arg Thr Gly Ile Tyr His Gly Gly Glu
350                 355                 360                 365

ccc tta tgt gac aat gtg aac act caa aga gta cct tgt tcc aat ccc   1155
Pro Leu Cys Asp Asn Val Asn Thr Gln Arg Val Pro Cys Ser Asn Pro
                370                 375                 380

agg tgg aat gaa tgg ctg aat tat gat ata tac att cct gat ctt cct   1203
Arg Trp Asn Glu Trp Leu Asn Tyr Asp Ile Tyr Ile Pro Asp Leu Pro
            385                 390                 395

cgt gct gct cga ctt tgc ctt tcc att tgc tct gtt aaa ggc cga aag   1251
Arg Ala Ala Arg Leu Cys Leu Ser Ile Cys Ser Val Lys Gly Arg Lys
        400                 405                 410

ggt gct aaa gag gaa cac tgt cca ttg gca tgg gga aat ata aac ttg   1299
Gly Ala Lys Glu Glu His Cys Pro Leu Ala Trp Gly Asn Ile Asn Leu
    415                 420                 425

ttt gat tac aca gac act cta gta tct gga aaa atg gct ttg aat ctt   1347
Phe Asp Tyr Thr Asp Thr Leu Val Ser Gly Lys Met Ala Leu Asn Leu
430                 435                 440                 445

tgg cca gta cct cat gga tta gaa gat ttg ctg aac cct att ggt gtt   1395
Trp Pro Val Pro His Gly Leu Glu Asp Leu Leu Asn Pro Ile Gly Val
                450                 455                 460

act gga tca aat cca aat aaa gaa act cca tgc tta gag ttg gag ttt   1443
Thr Gly Ser Asn Pro Asn Lys Glu Thr Pro Cys Leu Glu Leu Glu Phe
            465                 470                 475

gac tgg ttc agc agt gtg gta aag ttc cca gat atg tca gtg att gaa   1491
Asp Trp Phe Ser Ser Val Val Lys Phe Pro Asp Met Ser Val Ile Glu
        480                 485                 490

gag cat gcc aat tgg tct gta tcc cga gaa gca gga ttt agc tat tcc   1539
Glu His Ala Asn Trp Ser Val Ser Arg Glu Ala Gly Phe Ser Tyr Ser
    495                 500                 505

cac gca gga ctg agt aac aga cta gct aga gac aat gaa tta agg gaa   1587
His Ala Gly Leu Ser Asn Arg Leu Ala Arg Asp Asn Glu Leu Arg Glu
510                 515                 520                 525

aat gac aaa gaa cag ctc aaa gca att tct aca cga gat cct ctc tct   1635
Asn Asp Lys Glu Gln Leu Lys Ala Ile Ser Thr Arg Asp Pro Leu Ser
                530                 535                 540

gaa atc act gag cag gag aaa gat ttt cta tgg agt cac aga cac tat   1683
Glu Ile Thr Glu Gln Glu Lys Asp Phe Leu Trp Ser His Arg His Tyr
            545                 550                 555

tgt gta act atc ccc gaa att cta ccc aaa ttg ctt ctg tct gtt aaa   1731
Cys Val Thr Ile Pro Glu Ile Leu Pro Lys Leu Leu Leu Ser Val Lys
        560                 565                 570

tgg aat tct aga gat gaa gta gcc cag atg tat tgc ttg gta aaa gat   1779
Trp Asn Ser Arg Asp Glu Val Ala Gln Met Tyr Cys Leu Val Lys Asp
    575                 580                 585
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tgg cct cca atc aaa cct gaa cag gct atg gaa ctt ctg gac tgt aat   1827
Trp Pro Pro Ile Lys Pro Glu Gln Ala Met Glu Leu Leu Asp Cys Asn
590                 595                 600                 605

tac cca gat cct atg gtt cga ggt ttt gct gtt cgg tgc ttg gaa aaa   1875
Tyr Pro Asp Pro Met Val Arg Gly Phe Ala Val Arg Cys Leu Glu Lys
                610                 615                 620

tat tta aca gat gac aaa ctt tct cag tat tta att cag cta gta cag   1923
Tyr Leu Thr Asp Asp Lys Leu Ser Gln Tyr Leu Ile Gln Leu Val Gln
            625                 630                 635

gtc cta aaa tat gaa caa tat ttg gat aac ttg ctt gtg aga ttt tta   1971
Val Leu Lys Tyr Glu Gln Tyr Leu Asp Asn Leu Leu Val Arg Phe Leu
        640                 645                 650

ctg aag aaa gca ttg act aat caa agg att ggg cac ttt ttc ttt tgg   2019
Leu Lys Lys Ala Leu Thr Asn Gln Arg Ile Gly His Phe Phe Phe Trp
    655                 660                 665

cat tta aaa tct gag atg cac aat aaa aca gtt agc cag agg ttt ggc   2067
His Leu Lys Ser Glu Met His Asn Lys Thr Val Ser Gln Arg Phe Gly
670                 675                 680                 685

ctg ctt ttg gag tcc tat tgt cgt gca tgt ggg atg tat ttg aag cac   2115
Leu Leu Leu Glu Ser Tyr Cys Arg Ala Cys Gly Met Tyr Leu Lys His
                690                 695                 700

ctg aat agg caa gtc gag gca atg gaa aag ctc att aac tta act gac   2163
Leu Asn Arg Gln Val Glu Ala Met Glu Lys Leu Ile Asn Leu Thr Asp
            705                 710                 715

att ctc aaa cag gag agg aag gat gaa aca caa aag gta cag atg aag   2211
Ile Leu Lys Gln Glu Arg Lys Asp Glu Thr Gln Lys Val Gln Met Lys
        720                 725                 730

ttt tta gtt gag caa atg agg cga cca gat ttc atg gat gcc cta cag   2259
Phe Leu Val Glu Gln Met Arg Arg Pro Asp Phe Met Asp Ala Leu Gln
    735                 740                 745

ggc ttg ctg tct cct cta aac cct gct cat caa cta gga aac ctc agg   2307
Gly Leu Leu Ser Pro Leu Asn Pro Ala His Gln Leu Gly Asn Leu Arg
750                 755                 760                 765

ctt aaa gag tgt cga att atg tct tct gca aaa agg cca ctg tgg ttg   2355
Leu Lys Glu Cys Arg Ile Met Ser Ser Ala Lys Arg Pro Leu Trp Leu
                770                 775                 780

aat tgg gag aac cca gac atc atg tca gag tta ctg ttt cag aac aat   2403
Asn Trp Glu Asn Pro Asp Ile Met Ser Glu Leu Leu Phe Gln Asn Asn
            785                 790                 795

gag atc atc ttt aaa aat ggg gat gat tta cgg caa gat atg cta aca   2451
Glu Ile Ile Phe Lys Asn Gly Asp Asp Leu Arg Gln Asp Met Leu Thr
        800                 805                 810

ctt caa att att cgt att atg gaa aat atc tgg caa aat caa ggt ctt   2499
Leu Gln Ile Ile Arg Ile Met Glu Asn Ile Trp Gln Asn Gln Gly Leu
    815                 820                 825
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gat ctt cga atg tta cct tat ggt tgt ctg tca atc ggt gac tgt gtg   2547
Asp Leu Arg Met Leu Pro Tyr Gly Cys Leu Ser Ile Gly Asp Cys Val
830                 835                 840                 845

gga ctt att gag gtg gtg cga aat tct cac act att atg caa att cag   2595
Gly Leu Ile Glu Val Val Arg Asn Ser His Thr Ile Met Gln Ile Gln
                850                 855                 860

tgc aaa ggc ggc ttg aaa ggt gca ctg cag ttc aac agc cac aca cta   2643
Cys Lys Gly Gly Leu Lys Gly Ala Leu Gln Phe Asn Ser His Thr Leu
            865                 870                 875

cat cag tgg ctc aaa gac aag aac aaa gga gaa ata tat gat gca gcc   2691
His Gln Trp Leu Lys Asp Lys Asn Lys Gly Glu Ile Tyr Asp Ala Ala
        880                 885                 890

att gac ctg ttt aca cgt tca tgt gct gga tac tgt gta gct acc ttc   2739
Ile Asp Leu Phe Thr Arg Ser Cys Ala Gly Tyr Cys Val Ala Thr Phe
    895                 900                 905

att ttg gga att gga gat cgt cac aat agt aac atc atg gtg aaa gac   2787
Ile Leu Gly Ile Gly Asp Arg His Asn Ser Asn Ile Met Val Lys Asp
910                 915                 920                 925

gat gga caa ctg ttt cat ata gat ttt gga cac ttt ttg gat cac aag   2835
Asp Gly Gln Leu Phe His Ile Asp Phe Gly His Phe Leu Asp His Lys
                930                 935                 940

aag aaa aaa ttt ggt tat aaa cga gaa cgt gtg cca ttt gtt ttg aca   2883
Lys Lys Lys Phe Gly Tyr Lys Arg Glu Arg Val Pro Phe Val Leu Thr
            945                 950                 955

cag gat ttc tta ata gtg att agt aaa gga gcc caa gaa tgc aca aag   2931
Gln Asp Phe Leu Ile Val Ile Ser Lys Gly Ala Gln Glu Cys Thr Lys
        960                 965                 970

aca aga gaa ttt gag agg ttt cag gag atg tgt tac aag gct tat cta   2979
Thr Arg Glu Phe Glu Arg Phe Gln Glu Met Cys Tyr Lys Ala Tyr Leu
    975                 980                 985

gct att cga cag cat gcc aat ctc ttc ata aat ctt ttc tca atg atg   3027
Ala Ile Arg Gln His Ala Asn Leu Phe Ile Asn Leu Phe Ser Met Met
990                 995                1000                1005

ctt ggc tct gga atg cca gaa cta caa tct ttt gat gac att gca tac   3075
Leu Gly Ser Gly Met Pro Glu Leu Gln Ser Phe Asp Asp Ile Ala Tyr
                1010                1015                1020

att cga aag acc cta gcc tta gat aaa act gag caa gag gct ttg gag   3123
Ile Arg Lys Thr Leu Ala Leu Asp Lys Thr Glu Gln Glu Ala Leu Glu
            1025                1030                1035

tat ttc atg aaa caa atg aat gat gca cat cat ggt ggc tgg aca aca   3171
Tyr Phe Met Lys Gln Met Asn Asp Ala His His Gly Gly Trp Thr Thr
        1040                1045                1050

aaa atg gat tgg atc ttc cac aca att aaa cag cat gca ttg aac tga   3219
Lys Met Asp Trp Ile Phe His Thr Ile Lys Gln His Ala Leu Asn
    1055                1060                1065

aagataactg agaaaatgaa agctcactct ggattccaca ctgcactgtt aataactctc 3279
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agcaggcaaa gaccgattgc ataggaattg cacaatccat gaacagcatt agatttacag 3339

caagaacaga aataaaatac tatataattt aaataatgta aacgcaaaca gggtttgata 3399

gcacttaaac tagttcattt caaaa 3424

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Pro Pro Arg Ile Leu Val Glu Cys Leu Leu Pro Asn Gly Met Ile Val
            20                  25                  30

Thr Leu Glu Cys Leu Arg Glu Ala Thr Leu Val Thr Ile Lys His Glu
        35                  40                  45

Leu Phe Lys Glu Ala Arg Lys Tyr Pro Leu His Gln Leu Leu Gln Asp
    50                  55                  60

Glu Ser Ser Tyr Ile Phe Val Ser Val Thr Gln Glu Ala Glu Arg Glu
65                  70                  75                  80

Glu Phe Phe Asp Glu Thr Arg Arg Leu Cys Asp Leu Arg Leu Phe Gln
                85                  90                  95

Pro Phe Leu Lys Val Ile Glu Pro Val Gly Asn Arg Glu Glu Lys Ile
            100                 105                 110

Leu Asn Arg Glu Ile Gly Phe Ala Ile Gly Met Pro Val Cys Glu Phe
        115                 120                 125

Asp Met Val Lys Asp Pro Glu Val Gln Asp Phe Arg Arg Asn Ile Leu
    130                 135                 140

Asn Val Cys Lys Glu Ala Val Asp Leu Arg Asp Leu Asn Ser Pro His
145                 150                 155                 160

Ser Arg Ala Met Tyr Val Tyr Pro Pro His Val Glu Ser Ser Pro Glu
                165                 170                 175

Leu Pro Lys His Ile Tyr Asn Lys Leu Asp Arg Gly Gln Ile Ile Val
            180                 185                 190

Val Ile Trp Val Ile Val Ser Pro Asn Asn Asp Lys Gln Lys Tyr Thr
        195                 200                 205

Leu Lys Ile Asn His Asp Cys Val Pro Glu Gln Val Ile Ala Glu Ala
    210                 215                 220

Ile Arg Lys Lys Thr Arg Ser Met Leu Leu Ser Ser Glu Gln Leu Lys
225                 230                 235                 240

Leu Cys Val Leu Glu Tyr Gln Gly Lys Tyr Ile Leu Lys Val Cys Gly
                245                 250                 255
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Cys Asp Glu Tyr Phe Leu Glu Lys Tyr Pro Leu Ser Gln Tyr Lys Tyr
260 265 270

Ile Arg Ser Cys Ile Met Leu Gly Arg Met Pro Asn Leu Lys Met Met
275 280 285

Ala Lys Glu Ser Leu Tyr Ser Gln Leu Pro Met Asp Cys Phe Thr Met
290 295 300

Pro Ser Tyr Ser Arg Arg Ile Ser Thr Ala Thr Pro Tyr Met Asn Gly
305 310 315 320

Glu Thr Ser Thr Lys Ser Leu Trp Val Ile Asn Arg Ala Leu Arg Ile
325 330 335

Lys Ile Leu Cys Ala Thr Tyr Val Asn Leu Asn Ile Arg Asp Ile Asp
340 345 350

Lys Ile Tyr Val Arg Thr Gly Ile Tyr His Gly Gly Glu Pro Leu Cys
355 360 365

Asp Asn Val Asn Thr Gln Arg Val Pro Cys Ser Asn Pro Arg Trp Asn
370 375 380

Glu Trp Leu Asn Tyr Asp Ile Tyr Ile Pro Asp Leu Pro Arg Ala Ala
385 390 395 400

Arg Leu Cys Leu Ser Ile Cys Ser Val Lys Gly Arg Lys Gly Ala Lys
405 410 415

Glu Glu His Cys Pro Leu Ala Trp Gly Asn Ile Asn Leu Phe Asp Tyr
420 425 430

Thr Asp Thr Leu Val Ser Gly Lys Met Ala Leu Asn Leu Trp Pro Val
435 440 445

Pro His Gly Leu Glu Asp Leu Leu Asn Pro Ile Gly Val Thr Gly Ser
450 455 460

Asn Pro Asn Lys Glu Thr Pro Cys Leu Glu Leu Glu Phe Asp Trp Phe
465 470 475 480

Ser Ser Val Val Lys Phe Pro Asp Met Ser Val Ile Glu Glu His Ala
485 490 495

Asn Trp Ser Val Ser Arg Glu Ala Gly Phe Ser Tyr Ser His Ala Gly
500 505 510

Leu Ser Asn Arg Leu Ala Arg Asp Asn Glu Leu Arg Glu Asn Asp Lys
515 520 525

Glu Gln Leu Lys Ala Ile Ser Thr Arg Asp Pro Leu Ser Glu Ile Thr
530 535 540

Glu Gln Glu Lys Asp Phe Leu Trp Ser His Arg His Tyr Cys Val Thr
545 550 555 560

Ile Pro Glu Ile Leu Pro Lys Leu Leu Leu Ser Val Lys Trp Asn Ser
565 570 575

Arg Asp Glu Val Ala Gln Met Tyr Cys Leu Val Lys Asp Trp Pro Pro
580 585 590

Ile Lys Pro Glu Gln Ala Met Glu Leu Leu Asp Cys Asn Tyr Pro Asp
595 600 605

Pro Met Val Arg Gly Phe Ala Val Arg Cys Leu Glu Lys Tyr Leu Thr
610 615 620

Asp Asp Lys Leu Ser Gln Tyr Leu Ile Gln Leu Val Gln Val Leu Lys
625 630 635 640

Tyr Glu Gln Tyr Leu Asp Asn Leu Leu Val Arg Phe Leu Leu Lys Lys
645 650 655

Ala Leu Thr Asn Gln Arg Ile Gly His Phe Phe Phe Trp His Leu Lys
660 665 670

Ser Glu Met His Asn Lys Thr Val Ser Gln Arg Phe Gly Leu Leu Leu
675 680 685

Glu Ser Tyr Cys Arg Ala Cys Gly Met Tyr Leu Lys His Leu Asn Arg
690 695 700

Gln Val Glu Ala Met Glu Lys Leu Ile Asn Leu Thr Asp Ile Leu Lys
705 710 715 720

Gln Glu Arg Lys Asp Glu Thr Gln Lys Val Gln Met Lys Phe Leu Val
725 730 735

Glu Gln Met Arg Arg Pro Asp Phe Met Asp Ala Leu Gln Gly Leu Leu
740 745 750

Ser Pro Leu Asn Pro Ala His Gln Leu Gly Asn Leu Arg Leu Lys Glu
755 760 765

Cys Arg Ile Met Ser Ser Ala Lys Arg Pro Leu Trp Leu Asn Trp Glu
770 775 780

Asn Pro Asp Ile Met Ser Glu Leu Leu Phe Gln Asn Asn Glu Ile Ile
785 790 795 800

Phe Lys Asn Gly Asp Asp Leu Arg Gln Asp Met Leu Thr Leu Gln Ile
805 810 815

Ile Arg Ile Met Glu Asn Ile Trp Gln Asn Gln Gly Leu Asp Leu Arg
820 825 830

Met Leu Pro Tyr Gly Cys Leu Ser Ile Gly Asp Cys Val Gly Leu Ile
835 840 845

Glu Val Val Arg Asn Ser His Thr Ile Met Gln Ile Gln Cys Lys Gly
850 855 860

Gly Leu Lys Gly Ala Leu Gln Phe Asn Ser His Thr Leu His Gln Trp
865 870 875 880

Leu Lys Asp Lys Asn Lys Gly Glu Ile Tyr Asp Ala Ala Ile Asp Leu
885 890 895

Phe Thr Arg Ser Cys Ala Gly Tyr Cys Val Ala Thr Phe Ile Leu Gly
900 905 910

Ile Gly Asp Arg His Asn Ser Asn Ile Met Val Lys Asp Asp Gly Gln
915 920 925

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Leu Phe His Ile Asp Phe Gly His Phe Leu Asp His Lys Lys Lys Lys
    930                 935                 940

Phe Gly Tyr Lys Arg Glu Arg Val Pro Phe Val Leu Thr Gln Asp Phe
945                 950                 955                 960

Leu Ile Val Ile Ser Lys Gly Ala Gln Glu Cys Thr Lys Thr Arg Glu
                965                 970                 975

Phe Glu Arg Phe Gln Glu Met Cys Tyr Lys Ala Tyr Leu Ala Ile Arg
            980                 985                 990

Gln His Ala Asn Leu Phe Ile Asn Leu Phe Ser Met Met Leu Gly Ser
        995                1000                1005

Gly Met Pro Glu Leu Gln Ser Phe Asp Asp Ile Ala Tyr Ile Arg Lys
   1010                1015                1020

Thr Leu Ala Leu Asp Lys Thr Glu Gln Glu Ala Leu Glu Tyr Phe Met
1025                1030                1035                1040

Lys Gln Met Asn Asp Ala His His Gly Gly Trp Thr Thr Lys Met Asp
                1045                1050                1055

Trp Ile Phe His Thr Ile Lys Gln His Ala Leu Asn
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Met Cys Phe Ser Phe Ile Met Pro Pro Ala Met Ala Asp Ile Leu Asp
  1               5                  10                  15

atc tgg gcg gtg gat tca cag ata gca tct gat ggc tcc ata cct gtg   96
Ile Trp Ala Val Asp Ser Gln Ile Ala Ser Asp Gly Ser Ile Pro Val
             20                  25                  30

gat ttc ctt ttg ccc act ggg att tat atc cag ttg gag gta cct cgg   144
Asp Phe Leu Leu Pro Thr Gly Ile Tyr Ile Gln Leu Glu Val Pro Arg
         35                  40                  45

gaa gct acc att tct tat att aag cag atg tta tgg aag caa gtt cac   192
Glu Ala Thr Ile Ser Tyr Ile Lys Gln Met Leu Trp Lys Gln Val His
     50                  55                  60

aat tac cca atg ttc aac ctc ctt atg gat att gac tcc tat atg ttt   240
Asn Tyr Pro Met Phe Asn Leu Leu Met Asp Ile Asp Ser Tyr Met Phe
 65                  70                  75                  80
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gca tgt gtg aat cag act gct gta tat gag gag ctt gaa gat gaa aca   288
Ala Cys Val Asn Gln Thr Ala Val Tyr Glu Glu Leu Glu Asp Glu Thr
                85                  90                  95

cga aga ctc tgt gat gtc aga cct ttt ctt cca gtt ctc aaa tta gtg   336
Arg Arg Leu Cys Asp Val Arg Pro Phe Leu Pro Val Leu Lys Leu Val
            100                 105                 110

aca aga agt tgt gac cca ggg gaa aaa tta gac tca aaa att gga gtc   384
Thr Arg Ser Cys Asp Pro Gly Glu Lys Leu Asp Ser Lys Ile Gly Val
        115                 120                 125

ctt ata gga aaa ggt ctg cat gaa ttt gat tcc ttg aag gat cct gaa   432
Leu Ile Gly Lys Gly Leu His Glu Phe Asp Ser Leu Lys Asp Pro Glu
    130                 135                 140

gta aat gaa ttt cga aga aaa atg cgc aaa ttc agc gag gaa aaa atc   480
Val Asn Glu Phe Arg Arg Lys Met Arg Lys Phe Ser Glu Glu Lys Ile
145                 150                 155                 160

ctg tca ctt gtg gga ttg tct tgg atg gac tgg cta aaa caa aca tat   528
Leu Ser Leu Val Gly Leu Ser Trp Met Asp Trp Leu Lys Gln Thr Tyr
                165                 170                 175

cca cca gag cat gaa cca tcc atc cct gaa aac tta gaa gat aaa ctt   576
Pro Pro Glu His Glu Pro Ser Ile Pro Glu Asn Leu Glu Asp Lys Leu
            180                 185                 190

tat ggg gga aag ctc atc gta gct gtt cat ttt gaa aac tgc cag gac   624
Tyr Gly Gly Lys Leu Ile Val Ala Val His Phe Glu Asn Cys Gln Asp
        195                 200                 205

gtg ttt agc ttt caa gtg tct cct aat atg aat cct atc aaa gta aat   672
Val Phe Ser Phe Gln Val Ser Pro Asn Met Asn Pro Ile Lys Val Asn
    210                 215                 220

gaa ttg gca atc caa aaa cgt ttg act att cat ggg aag gaa gat gaa   720
Glu Leu Ala Ile Gln Lys Arg Leu Thr Ile His Gly Lys Glu Asp Glu
225                 230                 235                 240

gtt agc ccc tat gat tat gtg ttg caa gtc agc ggg aga gta gaa tat   768
Val Ser Pro Tyr Asp Tyr Val Leu Gln Val Ser Gly Arg Val Glu Tyr
                245                 250                 255

gtt ttt ggt gat cat cca cta att cag ttc cag tat atc cgg aac tgt   816
Val Phe Gly Asp His Pro Leu Ile Gln Phe Gln Tyr Ile Arg Asn Cys
            260                 265                 270

gtg atg aac aga gcc ctg ccc cat ttt ata ctt gtg gaa tgc tgc aag   864
Val Met Asn Arg Ala Leu Pro His Phe Ile Leu Val Glu Cys Cys Lys
        275                 280                 285

atc aag aaa atg tat gaa caa gaa atg att gcc ata gag gct gcc ata   912
Ile Lys Lys Met Tyr Glu Gln Glu Met Ile Ala Ile Glu Ala Ala Ile
    290                 295                 300

aat cga aat tca tct aat ctt cct ctt cca tta cca cca aag aaa aca   960
Asn Arg Asn Ser Ser Asn Leu Pro Leu Pro Leu Pro Pro Lys Lys Thr
305                 310                 315                 320
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cga att att tct cat gtt tgg gaa aat aac aac cct ttc caa att gtc   1008
Arg Ile Ile Ser His Val Trp Glu Asn Asn Asn Pro Phe Gln Ile Val
                325                 330                 335

ttg gtt aag gga aat aaa ctt aac aca gag gaa act gta aaa gtt cat   1056
Leu Val Lys Gly Asn Lys Leu Asn Thr Glu Glu Thr Val Lys Val His
            340                 345                 350

gtc agg gct ggt ctt ttt cat ggt act gag ctc ctg tgt aaa acc atc   1104
Val Arg Ala Gly Leu Phe His Gly Thr Glu Leu Leu Cys Lys Thr Ile
        355                 360                 365

gta agc tca gag gta tca ggg aaa aat gat cat att tgg aat gaa cca   1152
Val Ser Ser Glu Val Ser Gly Lys Asn Asp His Ile Trp Asn Glu Pro
    370                 375                 380

ctg gaa ttt gat att aat att tgt gac tta cca aga atg gct cga tta   1200
Leu Glu Phe Asp Ile Asn Ile Cys Asp Leu Pro Arg Met Ala Arg Leu
385                 390                 395                 400

tgt ttt gct gtt tat gca gtt ttg gat aaa gta aaa acg aag aaa tca   1248
Cys Phe Ala Val Tyr Ala Val Leu Asp Lys Val Lys Thr Lys Lys Ser
                405                 410                 415

acg aaa act att aat ccc tct aaa tat cag acc atc agg aaa gct gga   1296
Thr Lys Thr Ile Asn Pro Ser Lys Tyr Gln Thr Ile Arg Lys Ala Gly
            420                 425                 430

aaa gtg cat tat cct gta gcg tgg gta aat acg atg gtt ttt gac ttt   1344
Lys Val His Tyr Pro Val Ala Trp Val Asn Thr Met Val Phe Asp Phe
        435                 440                 445

aaa gga caa ttg aga act gga gac ata ata tta cac agc tgg tct tca   1392
Lys Gly Gln Leu Arg Thr Gly Asp Ile Ile Leu His Ser Trp Ser Ser
    450                 455                 460

ttt cct gat gaa ctc gaa gaa atg ttg aat cca atg gga act gtt caa   1440
Phe Pro Asp Glu Leu Glu Glu Met Leu Asn Pro Met Gly Thr Val Gln
465                 470                 475                 480

aca aat cca tat act gaa aat gca aca gct ttg cat gtt aaa ttt cca   1488
Thr Asn Pro Tyr Thr Glu Asn Ala Thr Ala Leu His Val Lys Phe Pro
                485                 490                 495

gag aat aaa aaa caa cct tat tat tac cct ccc ttc gat aag att att   1536
Glu Asn Lys Lys Gln Pro Tyr Tyr Tyr Pro Pro Phe Asp Lys Ile Ile
            500                 505                 510

gaa aag gca gct gag att gca agc agt gat agt gct aat gtg tca agt   1584
Glu Lys Ala Ala Glu Ile Ala Ser Ser Asp Ser Ala Asn Val Ser Ser
        515                 520                 525

cga ggt gga aaa aag ttt ctt cct gta ttg aaa gaa atc ttg gac agg   1632
Arg Gly Gly Lys Lys Phe Leu Pro Val Leu Lys Glu Ile Leu Asp Arg
    530                 535                 540

gat ccc ttg tct caa ctg tgt gaa aat gaa atg gat ctt att tgg act   1680
Asp Pro Leu Ser Gln Leu Cys Glu Asn Glu Met Asp Leu Ile Trp Thr
545                 550                 555                 560
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ttg cga caa gac tgc cga gag att ttc cca caa tca ctg cca aaa tta   1728
Leu Arg Gln Asp Cys Arg Glu Ile Phe Pro Gln Ser Leu Pro Lys Leu
                565                 570                 575

ctg ctg tca atc aag tgg aat aaa ctt gag gat gtt gct cag ctt cag   1776
Leu Leu Ser Ile Lys Trp Asn Lys Leu Glu Asp Val Ala Gln Leu Gln
            580                 585                 590

gcg ctg ctt cag att tgg cct aaa ctg ccc ccc cgg gag gcc cta gag   1824
Ala Leu Leu Gln Ile Trp Pro Lys Leu Pro Pro Arg Glu Ala Leu Glu
        595                 600                 605

ctt ctg gat ttc aac tat cca gac cag tac gtt cga gaa tat gct gta   1872
Leu Leu Asp Phe Asn Tyr Pro Asp Gln Tyr Val Arg Glu Tyr Ala Val
    610                 615                 620

ggc tgc ctg cga cag atg agt gat gaa gaa ctt tct caa tat ctt tta   1920
Gly Cys Leu Arg Gln Met Ser Asp Glu Glu Leu Ser Gln Tyr Leu Leu
625                 630                 635                 640

caa ctg gtg caa gtg tta aaa tat gag cct ttt ctt gat tgt gcc ctc   1968
Gln Leu Val Gln Val Leu Lys Tyr Glu Pro Phe Leu Asp Cys Ala Leu
                645                 650                 655

tct aga ttc cta tta gaa aga gca ctt ggt aat cgg agg ata ggg cag   2016
Ser Arg Phe Leu Leu Glu Arg Ala Leu Gly Asn Arg Arg Ile Gly Gln
            660                 665                 670

ttt cta ttt tgg cat ctt agg tca gaa gtg cac att cct gct gtc tca   2064
Phe Leu Phe Trp His Leu Arg Ser Glu Val His Ile Pro Ala Val Ser
        675                 680                 685

gta caa ttt ggt gtc atc ctt gaa gca tac tgc cgg gga agt gtg ggg   2112
Val Gln Phe Gly Val Ile Leu Glu Ala Tyr Cys Arg Gly Ser Val Gly
    690                 695                 700

cac atg aaa gtg ctt tct aag cag gtt gaa gca ctc aat aag tta aaa   2160
His Met Lys Val Leu Ser Lys Gln Val Glu Ala Leu Asn Lys Leu Lys
705                 710                 715                 720

act tta aat agt tta atc aaa ctg aat gcc gtg aag tta aac aga gcc   2208
Thr Leu Asn Ser Leu Ile Lys Leu Asn Ala Val Lys Leu Asn Arg Ala
                725                 730                 735

aaa ggg aag gag gcc atg cat acc tgt tta aaa cag agt gct tac cgg   2256
Lys Gly Lys Glu Ala Met His Thr Cys Leu Lys Gln Ser Ala Tyr Arg
            740                 745                 750

gaa gcc ctc tct gac ctg cag tca ccc ctg aac cca tgt gtt atc ctc   2304
Glu Ala Leu Ser Asp Leu Gln Ser Pro Leu Asn Pro Cys Val Ile Leu
        755                 760                 765

tca gaa ctc tat gtt gaa aag tgc aaa tac atg gat tcc aaa atg aag   2352
Ser Glu Leu Tyr Val Glu Lys Cys Lys Tyr Met Asp Ser Lys Met Lys
    770                 775                 780

cct ttg tgg ctg gta tac aat aac aag gta ttt ggt gag gat tca gtt   2400
Pro Leu Trp Leu Val Tyr Asn Asn Lys Val Phe Gly Glu Asp Ser Val
785                 790                 795                 800
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gga gtg att ttt aaa aat ggt gat gat tta cga cag gat atg ttg aca   2448
Gly Val Ile Phe Lys Asn Gly Asp Asp Leu Arg Gln Asp Met Leu Thr
                805                 810                 815

ctc caa atg ttg cgc ttg atg gat tta ctc tgg aaa gaa gct ggt ttg   2496
Leu Gln Met Leu Arg Leu Met Asp Leu Leu Trp Lys Glu Ala Gly Leu
            820                 825                 830

gat ctt cgg atg ttg cct tat ggc tgt tta gca aca gga gat cgc tct   2544
Asp Leu Arg Met Leu Pro Tyr Gly Cys Leu Ala Thr Gly Asp Arg Ser
        835                 840                 845

ggc ctc att gaa gtt gtg agc acc tct gaa aca att gct gac att cag   2592
Gly Leu Ile Glu Val Val Ser Thr Ser Glu Thr Ile Ala Asp Ile Gln
    850                 855                 860

ctg aac agt agc aat gtg gct gct gca gca gcc ttc aac aaa gat gcc   2640
Leu Asn Ser Ser Asn Val Ala Ala Ala Ala Ala Phe Asn Lys Asp Ala
865                 870                 875                 880

ctt ctg aac tgg ctt aaa gaa tac aac tct ggg gat gac ctg gac cga   2688
Leu Leu Asn Trp Leu Lys Glu Tyr Asn Ser Gly Asp Asp Leu Asp Arg
                885                 890                 895

gcc att gag gaa ttt aca ctg tcc tgt gct ggc tac tgt gta gct tct   2736
Ala Ile Glu Glu Phe Thr Leu Ser Cys Ala Gly Tyr Cys Val Ala Ser
            900                 905                 910

tat gtc ctt ggg att ggt gac aga cat agt gac aac atc atg gtc aaa   2784
Tyr Val Leu Gly Ile Gly Asp Arg His Ser Asp Asn Ile Met Val Lys
        915                 920                 925

aaa act ggc cag ctc ttc cac att gac ttt gga cat att ctt gga aat   2832
Lys Thr Gly Gln Leu Phe His Ile Asp Phe Gly His Ile Leu Gly Asn
    930                 935                 940

ttc aaa tct aag ttt ggc att aaa agg gag cga gtg cct ttt att ctt   2880
Phe Lys Ser Lys Phe Gly Ile Lys Arg Glu Arg Val Pro Phe Ile Leu
945                 950                 955                 960

acc tat gat ttc atc cat gtc att caa caa gga aaa aca gga aat aca   2928
Thr Tyr Asp Phe Ile His Val Ile Gln Gln Gly Lys Thr Gly Asn Thr
                965                 970                 975

gaa aag ttt ggc cgg ttc cgc cag tgt tgt gag gat gca tat ctg att   2976
Glu Lys Phe Gly Arg Phe Arg Gln Cys Cys Glu Asp Ala Tyr Leu Ile
            980                 985                 990

tta cga cgg cat ggg aat ctc ttc atc act ctc ttt gcg ctg atg ttg   3024
Leu Arg Arg His Gly Asn Leu Phe Ile Thr Leu Phe Ala Leu Met Leu
        995                1000                1005

act gca ggg ctt cct gaa ctc aca tca gtc aaa gat ata cag tat ctt   3072
Thr Ala Gly Leu Pro Glu Leu Thr Ser Val Lys Asp Ile Gln Tyr Leu
   1010                1015                1020

aag gac tct ctt gca tta ggg aag agt gaa gaa gaa gca ctc aaa cag   3120
Lys Asp Ser Leu Ala Leu Gly Lys Ser Glu Glu Glu Ala Leu Lys Gln
1025                1030                1035                1040
```

```
ttt aag caa aaa ttt gat gag gcg ctc agg gaa agc tgg act act aaa   3168
Phe Lys Gln Lys Phe Asp Glu Ala Leu Arg Glu Ser Trp Thr Thr Lys
                1045                1050                1055

gtg aac tgg atg gcc cac aca gtt cgg aaa gac tac aga tct taa       3213
Val Asn Trp Met Ala His Thr Val Arg Lys Asp Tyr Arg Ser
            1060                1065                1070
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Met Cys Phe Ser Phe Ile Met Pro Pro Ala Met Ala Asp Ile Leu Asp
  1               5                  10                  15

Ile Trp Ala Val Asp Ser Gln Ile Ala Ser Asp Gly Ser Ile Pro Val
             20                  25                  30

Asp Phe Leu Leu Pro Thr Gly Ile Tyr Ile Gln Leu Glu Val Pro Arg
         35                  40                  45

Glu Ala Thr Ile Ser Tyr Ile Lys Gln Met Leu Trp Lys Gln Val His
     50                  55                  60

Asn Tyr Pro Met Phe Asn Leu Leu Met Asp Ile Asp Ser Tyr Met Phe
 65                  70                  75                  80

Ala Cys Val Asn Gln Thr Ala Val Tyr Glu Glu Leu Glu Asp Glu Thr
                85                  90                  95

Arg Arg Leu Cys Asp Val Arg Pro Phe Leu Pro Val Leu Lys Leu Val
            100                 105                 110

Thr Arg Ser Cys Asp Pro Gly Glu Lys Leu Asp Ser Lys Ile Gly Val
        115                 120                 125

Leu Ile Gly Lys Gly Leu His Glu Phe Asp Ser Leu Lys Asp Pro Glu
    130                 135                 140

Val Asn Glu Phe Arg Arg Lys Met Arg Lys Phe Ser Glu Glu Lys Ile
145                 150                 155                 160

Leu Ser Leu Val Gly Leu Ser Trp Met Asp Trp Leu Lys Gln Thr Tyr
                165                 170                 175

Pro Pro Glu His Glu Pro Ser Ile Pro Glu Asn Leu Glu Asp Lys Leu
            180                 185                 190

Tyr Gly Gly Lys Leu Ile Val Ala Val His Phe Glu Asn Cys Gln Asp
        195                 200                 205

Val Phe Ser Phe Gln Val Ser Pro Asn Met Asn Pro Ile Lys Val Asn
    210                 215                 220

Glu Leu Ala Ile Gln Lys Arg Leu Thr Ile His Gly Lys Glu Asp Glu
225                 230                 235                 240

Val Ser Pro Tyr Asp Tyr Val Leu Gln Val Ser Gly Arg Val Glu Tyr
                245                 250                 255
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Val Phe Gly Asp His Pro Leu Ile Gln Phe Gln Tyr Ile Arg Asn Cys
260 265 270

Val Met Asn Arg Ala Leu Pro His Phe Ile Leu Val Glu Cys Cys Lys
275 280 285

Ile Lys Lys Met Tyr Glu Gln Glu Met Ile Ala Ile Glu Ala Ala Ile
290 295 300

Asn Arg Asn Ser Ser Asn Leu Pro Leu Pro Leu Pro Pro Lys Lys Thr
305 310 315 320

Arg Ile Ile Ser His Val Trp Glu Asn Asn Asn Pro Phe Gln Ile Val
325 330 335

Leu Val Lys Gly Asn Lys Leu Asn Thr Glu Glu Thr Val Lys Val His
340 345 350

Val Arg Ala Gly Leu Phe His Gly Thr Glu Leu Leu Cys Lys Thr Ile
355 360 365

Val Ser Ser Glu Val Ser Gly Lys Asn Asp His Ile Trp Asn Glu Pro
370 375 380

Leu Glu Phe Asp Ile Asn Ile Cys Asp Leu Pro Arg Met Ala Arg Leu
385 390 395 400

Cys Phe Ala Val Tyr Ala Val Leu Asp Lys Val Lys Thr Lys Lys Ser
405 410 415

Thr Lys Thr Ile Asn Pro Ser Lys Tyr Gln Thr Ile Arg Lys Ala Gly
420 425 430

Lys Val His Tyr Pro Val Ala Trp Val Asn Thr Met Val Phe Asp Phe
435 440 445

Lys Gly Gln Leu Arg Thr Gly Asp Ile Ile Leu His Ser Trp Ser Ser
450 455 460

Phe Pro Asp Glu Leu Glu Glu Met Leu Asn Pro Met Gly Thr Val Gln
465 470 475 480

Thr Asn Pro Tyr Thr Glu Asn Ala Thr Ala Leu His Val Lys Phe Pro
485 490 495

Glu Asn Lys Lys Gln Pro Tyr Tyr Tyr Pro Pro Phe Asp Lys Ile Ile
500 505 510

Glu Lys Ala Ala Glu Ile Ala Ser Ser Asp Ser Ala Asn Val Ser Ser
515 520 525

Arg Gly Gly Lys Lys Phe Leu Pro Val Leu Lys Glu Ile Leu Asp Arg
530 535 540

Asp Pro Leu Ser Gln Leu Cys Glu Asn Glu Met Asp Leu Ile Trp Thr
545 550 555 560

Leu Arg Gln Asp Cys Arg Glu Ile Phe Pro Gln Ser Leu Pro Lys Leu
565 570 575

Leu Leu Ser Ile Lys Trp Asn Lys Leu Glu Asp Val Ala Gln Leu Gln
580 585 590

Ala Leu Leu Gln Ile Trp Pro Lys Leu Pro Pro Arg Glu Ala Leu Glu
595 600 605

Leu Leu Asp Phe Asn Tyr Pro Asp Gln Tyr Val Arg Glu Tyr Ala Val
610 615 620

Gly Cys Leu Arg Gln Met Ser Asp Glu Glu Leu Ser Gln Tyr Leu Leu
625 630 635 640

Gln Leu Val Gln Val Leu Lys Tyr Glu Pro Phe Leu Asp Cys Ala Leu
645 650 655

Ser Arg Phe Leu Leu Glu Arg Ala Leu Gly Asn Arg Arg Ile Gly Gln
660 665 670

Phe Leu Phe Trp His Leu Arg Ser Glu Val His Ile Pro Ala Val Ser
675 680 685

Val Gln Phe Gly Val Ile Leu Glu Ala Tyr Cys Arg Gly Ser Val Gly
690 695 700

His Met Lys Val Leu Ser Lys Gln Val Glu Ala Leu Asn Lys Leu Lys
705 710 715 720

Thr Leu Asn Ser Leu Ile Lys Leu Asn Ala Val Lys Leu Asn Arg Ala
725 730 735

Lys Gly Lys Glu Ala Met His Thr Cys Leu Lys Gln Ser Ala Tyr Arg
740 745 750

Glu Ala Leu Ser Asp Leu Gln Ser Pro Leu Asn Pro Cys Val Ile Leu
755 760 765

Ser Glu Leu Tyr Val Glu Lys Cys Lys Tyr Met Asp Ser Lys Met Lys
770 775 780

Pro Leu Trp Leu Val Tyr Asn Asn Lys Val Phe Gly Glu Asp Ser Val
785 790 795 800

Gly Val Ile Phe Lys Asn Gly Asp Asp Leu Arg Gln Asp Met Leu Thr
805 810 815

Leu Gln Met Leu Arg Leu Met Asp Leu Leu Trp Lys Glu Ala Gly Leu
820 825 830

Asp Leu Arg Met Leu Pro Tyr Gly Cys Leu Ala Thr Gly Asp Arg Ser
835 840 845

Gly Leu Ile Glu Val Val Ser Thr Ser Glu Thr Ile Ala Asp Ile Gln
850 855 860

Leu Asn Ser Ser Asn Val Ala Ala Ala Ala Ala Phe Asn Lys Asp Ala
865 870 875 880

Leu Leu Asn Trp Leu Lys Glu Tyr Asn Ser Gly Asp Asp Leu Asp Arg
885 890 895

Ala Ile Glu Glu Phe Thr Leu Ser Cys Ala Gly Tyr Cys Val Ala Ser
900 905 910

Tyr Val Leu Gly Ile Gly Asp Arg His Ser Asp Asn Ile Met Val Lys
915 920 925

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Lys Thr Gly Gln Leu Phe His Ile Asp Phe Gly His Ile Leu Gly Asn
    930                 935                 940

Phe Lys Ser Lys Phe Gly Ile Lys Arg Glu Arg Val Pro Phe Ile Leu
945                 950                 955                 960

Thr Tyr Asp Phe Ile His Val Ile Gln Gln Gly Lys Thr Gly Asn Thr
                965                 970                 975

Glu Lys Phe Gly Arg Phe Arg Gln Cys Cys Glu Asp Ala Tyr Leu Ile
            980                 985                 990

Leu Arg Arg His Gly Asn Leu Phe Ile Thr Leu Phe Ala Leu Met Leu
        995                1000                1005

Thr Ala Gly Leu Pro Glu Leu Thr Ser Val Lys Asp Ile Gln Tyr Leu
   1010                1015                1020

Lys Asp Ser Leu Ala Leu Gly Lys Ser Glu Glu Glu Ala Leu Lys Gln
1025                1030                1035                1040

Phe Lys Gln Lys Phe Asp Glu Ala Leu Arg Glu Ser Trp Thr Thr Lys
                1045                1050                1055

Val Asn Trp Met Ala His Thr Val Arg Lys Asp Tyr Arg Ser
            1060                1065                1070
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gatgatacta ccaccgaatg tctgtgtttc attgtctagt ccaacctgta ttgtggatat 120

ctacaacgtt ccggcaatag ttttgcaggt gcatcacatt tttgtttttg ttttgggagg 180

aaaagggagg gcacggcagc caggcttcat attcctacaa gtgcatgctt caagattact 240

gtacttacag tgtttccaac atcttctcat aaaaggggaa agcttcatag cctcaaccat 300

gaaggaaacc agtcgcatag ggc atg gag ctg gag aac tat aaa cag ccc gtg 353
                          Met Glu Leu Glu Asn Tyr Lys Gln Pro Val
                            1               5                  10

gtg ctg aga gag gac aac tgc cga agg cgc cgg agg atg aag ccg cgc   401
Val Leu Arg Glu Asp Asn Cys Arg Arg Arg Arg Arg Met Lys Pro Arg
                15                  20                  25
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agt gct gcc agc ctg tcc tcc atg gag ctc atc ccc atc gag ttc gtg   449
Ser Ala Ala Ser Leu Ser Ser Met Glu Leu Ile Pro Ile Glu Phe Val
            30                  35                  40

ctg ccc acc agc cag cgc aaa tgc aag agc ccc gaa acg gcg ctg ctg   497
Leu Pro Thr Ser Gln Arg Lys Cys Lys Ser Pro Glu Thr Ala Leu Leu
        45                  50                  55

cac gtg gcc ggc cac ggc aac gtg gag cag atg aag gcc cag gtg tgg   545
His Val Ala Gly His Gly Asn Val Glu Gln Met Lys Ala Gln Val Trp
    60                  65                  70

ctg cga gcg ctg gag acc agc gtg gcg gcg gac ttc tac cac cgg ctg   593
Leu Arg Ala Leu Glu Thr Ser Val Ala Ala Asp Phe Tyr His Arg Leu
75                  80                  85                  90

gga ccg cat cac ttc ctc ctg ctc tat cag aag aag ggg cag tgg tac   641
Gly Pro His His Phe Leu Leu Leu Tyr Gln Lys Lys Gly Gln Trp Tyr
                95                  100                 105

gag atc tac gac aag tac cag gtg gtg cag act ctg gac tgc ctg cgc   689
Glu Ile Tyr Asp Lys Tyr Gln Val Val Gln Thr Leu Asp Cys Leu Arg
            110                 115                 120

tac tgg aag gcc acg cac cgg agc ccg ggc cag atc cac ctg gtg cag   737
Tyr Trp Lys Ala Thr His Arg Ser Pro Gly Gln Ile His Leu Val Gln
        125                 130                 135

cgg cac ccg ccc tcc gag gag tcc caa gcc ttc cag cgg cag ctc acg   785
Arg His Pro Pro Ser Glu Glu Ser Gln Ala Phe Gln Arg Gln Leu Thr
    140                 145                 150

gcg ctg att ggc tat gac gtc act gac gtc agc aac gtg cac gac gat   833
Ala Leu Ile Gly Tyr Asp Val Thr Asp Val Ser Asn Val His Asp Asp
155                 160                 165                 170

gag ctg gag ttc acg cgc cgt ggc ttg gtg acc ccg cgc atg gcg gag   881
Glu Leu Glu Phe Thr Arg Arg Gly Leu Val Thr Pro Arg Met Ala Glu
                175                 180                 185

gtg gcc agc cgc gac ccc aag ctc tac gcc atg cac ccg tgg gtg acg   929
Val Ala Ser Arg Asp Pro Lys Leu Tyr Ala Met His Pro Trp Val Thr
            190                 195                 200

tcc aag ccc ctc ccg gag tac ctg tgg aag aag att gcc aac aac tgc   977
Ser Lys Pro Leu Pro Glu Tyr Leu Trp Lys Lys Ile Ala Asn Asn Cys
        205                 210                 215

atc ttc atc gtc att cac cgc agc acc acc agc cag acc att aag gtc   1025
Ile Phe Ile Val Ile His Arg Ser Thr Thr Ser Gln Thr Ile Lys Val
    220                 225                 230

tca ccc gac gac acc ccc ggc gcc atc ctg cag agc ttc ttc acc aag   1073
Ser Pro Asp Asp Thr Pro Gly Ala Ile Leu Gln Ser Phe Phe Thr Lys
235                 240                 245                 250

atg gcc aag aag aaa tct ctg atg gat att ccc gaa agc caa agc gaa   1121
Met Ala Lys Lys Lys Ser Leu Met Asp Ile Pro Glu Ser Gln Ser Glu
                255                 260                 265
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cag gat ttt gtg ctg cgc gtc tgt ggc cgg gat gag tac ctg gtg ggc   1169
Gln Asp Phe Val Leu Arg Val Cys Gly Arg Asp Glu Tyr Leu Val Gly
            270                 275                 280

gaa acg ccc atc aaa aac ttc cag tgg gtg agg cac tgc ctc aag aac   1217
Glu Thr Pro Ile Lys Asn Phe Gln Trp Val Arg His Cys Leu Lys Asn
        285                 290                 295

gga gaa gag att cac gtg gta ctg gac acg cct cca gac ccg gcc cta   1265
Gly Glu Glu Ile His Val Val Leu Asp Thr Pro Pro Asp Pro Ala Leu
    300                 305                 310

gac gag gtg agg aag gaa gag tgg ccg ctg gtg gac gac tgc acg gga   1313
Asp Glu Val Arg Lys Glu Glu Trp Pro Leu Val Asp Asp Cys Thr Gly
315                 320                 325                 330

gtc acc ggc tac cat gag cag ctt acc atc cac ggc aag gac cac gag   1361
Val Thr Gly Tyr His Glu Gln Leu Thr Ile His Gly Lys Asp His Glu
                335                 340                 345

agt gtg ttc acc gtg tcc ctg tgg gac tgc gac cgc aag ttc agg gtc   1409
Ser Val Phe Thr Val Ser Leu Trp Asp Cys Asp Arg Lys Phe Arg Val
            350                 355                 360

aag atc aga ggc att gat atc ccc gtc ctg cct cgg aac acc gac ctc   1457
Lys Ile Arg Gly Ile Asp Ile Pro Val Leu Pro Arg Asn Thr Asp Leu
        365                 370                 375

aca gtt ttt gta gag gca aac atc cag cat ggg caa caa gtc ctt tgc   1505
Thr Val Phe Val Glu Ala Asn Ile Gln His Gly Gln Gln Val Leu Cys
    380                 385                 390

caa agg aga acc agc ccc aaa ccc ttc aca gag gag gtg ctg tgg aat   1553
Gln Arg Arg Thr Ser Pro Lys Pro Phe Thr Glu Glu Val Leu Trp Asn
395                 400                 405                 410

gtg tgg ctt gag ttc agt atc aaa atc aaa gac ttg ccc aaa ggg gct   1601
Val Trp Leu Glu Phe Ser Ile Lys Ile Lys Asp Leu Pro Lys Gly Ala
                415                 420                 425

cta ctg aac ctc cag atc tac tgc ggt aaa gct cca gca ctg tcc agc   1649
Leu Leu Asn Leu Gln Ile Tyr Cys Gly Lys Ala Pro Ala Leu Ser Ser
            430                 435                 440

aag gcc tct gca gag tcc ccc agt tct gag tcc aag ggc aaa gtt cgg   1697
Lys Ala Ser Ala Glu Ser Pro Ser Ser Glu Ser Lys Gly Lys Val Arg
        445                 450                 455

ctt ctc tat tat gtg aac ctg ctg ctg ata gac cac cgt ttc ctc ctg   1745
Leu Leu Tyr Tyr Val Asn Leu Leu Leu Ile Asp His Arg Phe Leu Leu
    460                 465                 470

cgc cgt gga gaa tac gtc ctc cac atg tgg cag ata tct ggg aag gga   1793
Arg Arg Gly Glu Tyr Val Leu His Met Trp Gln Ile Ser Gly Lys Gly
475                 480                 485                 490

gaa gac caa gga agc ttc aat gct gac aaa ctc acg tct gca act aac   1841
Glu Asp Gln Gly Ser Phe Asn Ala Asp Lys Leu Thr Ser Ala Thr Asn
                495                 500                 505
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cca gac aag gag aac tca atg tcc atc tcc att ctt ctg gac aat tac   1889
Pro Asp Lys Glu Asn Ser Met Ser Ile Ser Ile Leu Leu Asp Asn Tyr
            510                 515                 520

tgc cac ccg ata gcc ctg cct aag cat cag ccc acc cct gac ccg gaa   1937
Cys His Pro Ile Ala Leu Pro Lys His Gln Pro Thr Pro Asp Pro Glu
        525                 530                 535

ggg gac cgg gtt cga gca gaa atg ccc aac cag ctt cgc aag caa ttg   1985
Gly Asp Arg Val Arg Ala Glu Met Pro Asn Gln Leu Arg Lys Gln Leu
    540                 545                 550

gag gcg atc ata gcc act gat cca ctt aac cct ctc aca gca gag gac   2033
Glu Ala Ile Ile Ala Thr Asp Pro Leu Asn Pro Leu Thr Ala Glu Asp
555                 560                 565                 570

aaa gaa ttg ctc tgg cat ttt aga tac gaa agc ctt aag cac cca aaa   2081
Lys Glu Leu Leu Trp His Phe Arg Tyr Glu Ser Leu Lys His Pro Lys
                575                 580                 585

gca tat cct aag cta ttt agt tca gtg aaa tgg gga cag caa gaa att   2129
Ala Tyr Pro Lys Leu Phe Ser Ser Val Lys Trp Gly Gln Gln Glu Ile
            590                 595                 600

gtg gcc aaa aca tac caa ttg ttg gcc aga agg gaa gtc tgg gat caa   2177
Val Ala Lys Thr Tyr Gln Leu Leu Ala Arg Arg Glu Val Trp Asp Gln
        605                 610                 615

agt gct ttg gat gtt ggg tta aca atg cag ctc ctg gac tgc aac ttc   2225
Ser Ala Leu Asp Val Gly Leu Thr Met Gln Leu Leu Asp Cys Asn Phe
    620                 625                 630

tca gat gaa aat gta aga gcc att gca gtt cag aaa ctg gag agc ttg   2273
Ser Asp Glu Asn Val Arg Ala Ile Ala Val Gln Lys Leu Glu Ser Leu
635                 640                 645                 650

gag gac gat gat gtt ctg cat tac ctt cta caa ttg gtc cag gct gtg   2321
Glu Asp Asp Asp Val Leu His Tyr Leu Leu Gln Leu Val Gln Ala Val
                655                 660                 665

aaa ttt gaa cca tac cat gat agc gcc ctt gcc aga ttt ctg ctg aag   2369
Lys Phe Glu Pro Tyr His Asp Ser Ala Leu Ala Arg Phe Leu Leu Lys
            670                 675                 680

cgt ggt tta aga aac aaa aga att ggt cac ttt ttg ttt tgg ttc ttg   2417
Arg Gly Leu Arg Asn Lys Arg Ile Gly His Phe Leu Phe Trp Phe Leu
        685                 690                 695

aga agt gag ata gcc cag tcc aga cac tat cag cag agg ttc gct gtg   2465
Arg Ser Glu Ile Ala Gln Ser Arg His Tyr Gln Gln Arg Phe Ala Val
    700                 705                 710

att ctg gaa gcc tat ctg agg ggc tgt ggc aca gcc atg ctg cac gac   2513
Ile Leu Glu Ala Tyr Leu Arg Gly Cys Gly Thr Ala Met Leu His Asp
715                 720                 725                 730

ttt acc caa caa gtc caa gta atc gag atg tta caa aaa gtc acc ctt   2561
Phe Thr Gln Gln Val Gln Val Ile Glu Met Leu Gln Lys Val Thr Leu
                735                 740                 745
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gat att aaa tcg ctc tct gct gaa aag tat gac gtc agt tcc caa gtt   2609
Asp Ile Lys Ser Leu Ser Ala Glu Lys Tyr Asp Val Ser Ser Gln Val
            750                 755                 760

att tca caa ctt aaa caa aag ctt gaa aac ctg cag aat tct caa ctc   2657
Ile Ser Gln Leu Lys Gln Lys Leu Glu Asn Leu Gln Asn Ser Gln Leu
        765                 770                 775

ccc gaa agc ttt aga gtt cca tat gat cct gga ctg aaa gca gga gcg   2705
Pro Glu Ser Phe Arg Val Pro Tyr Asp Pro Gly Leu Lys Ala Gly Ala
    780                 785                 790

ctg gca att gaa aaa tgt aaa gta atg gcc tcc aag aaa aaa cca cta   2753
Leu Ala Ile Glu Lys Cys Lys Val Met Ala Ser Lys Lys Lys Pro Leu
795                 800                 805                 810

tgg ctt gag ttt aaa tgt gcc gat cct aca gcc cta tca aat gaa aca   2801
Trp Leu Glu Phe Lys Cys Ala Asp Pro Thr Ala Leu Ser Asn Glu Thr
                815                 820                 825

att gga att atc ttt aaa cat ggt gat gat ctg cgc caa gac atg ctt   2849
Ile Gly Ile Ile Phe Lys His Gly Asp Asp Leu Arg Gln Asp Met Leu
            830                 835                 840

att tta cag att cta cga atc atg gag tct att tgg gag act gaa tct   2897
Ile Leu Gln Ile Leu Arg Ile Met Glu Ser Ile Trp Glu Thr Glu Ser
        845                 850                 855

ttg gat cta tgc ctc ctg cca tat ggt tgc att tca act ggt gac aaa   2945
Leu Asp Leu Cys Leu Leu Pro Tyr Gly Cys Ile Ser Thr Gly Asp Lys
    860                 865                 870

ata gga atg atc gag att gtg aaa gac gcc acg aca att gcc aaa att   2993
Ile Gly Met Ile Glu Ile Val Lys Asp Ala Thr Thr Ile Ala Lys Ile
875                 880                 885                 890

cag caa agc aca gtg ggc aac acg gga gca ttt aaa gat gaa gtc ctg   3041
Gln Gln Ser Thr Val Gly Asn Thr Gly Ala Phe Lys Asp Glu Val Leu
                895                 900                 905

aat cac tgg ctc aaa gaa aaa tcc cct act gaa gaa aag ttt cag gca   3089
Asn His Trp Leu Lys Glu Lys Ser Pro Thr Glu Glu Lys Phe Gln Ala
            910                 915                 920

gca gtg gag aga ttt gtt tat tcc tgt gca ggc tac tgt gtg gca acc   3137
Ala Val Glu Arg Phe Val Tyr Ser Cys Ala Gly Tyr Cys Val Ala Thr
        925                 930                 935

ttt gtt ctt gga ata ggc gac aga cac aat gac aat att atg atc acc   3185
Phe Val Leu Gly Ile Gly Asp Arg His Asn Asp Asn Ile Met Ile Thr
    940                 945                 950

gag aca gga aac cta ttt cat att gac ttc ggg cac att ctt ggg aat   3233
Glu Thr Gly Asn Leu Phe His Ile Asp Phe Gly His Ile Leu Gly Asn
955                 960                 965                 970

tac aaa agt ttc ctg ggc att aat aaa gag aga gtg cca ttt gtg cta   3281
Tyr Lys Ser Phe Leu Gly Ile Asn Lys Glu Arg Val Pro Phe Val Leu
                975                 980                 985
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acc cct gac ttc ctc ttt gtg atg gga act tct gga aag aag aca agc   3329
Thr Pro Asp Phe Leu Phe Val Met Gly Thr Ser Gly Lys Lys Thr Ser
            990                 995                1000

cca cac ttc cag aaa ttt cag gac atc tgt gtt aag gct tat cta gcc   3377
Pro His Phe Gln Lys Phe Gln Asp Ile Cys Val Lys Ala Tyr Leu Ala
       1005                1010                1015

ctt cgt cat cac aca aac cta ctg atc atc ctg ttc tcc atg atg ctg   3425
Leu Arg His His Thr Asn Leu Leu Ile Ile Leu Phe Ser Met Met Leu
   1020                1025                1030

atg aca gga atg ccc cag tta aca agc aaa gaa gac att gaa tat atc   3473
Met Thr Gly Met Pro Gln Leu Thr Ser Lys Glu Asp Ile Glu Tyr Ile
1035                1040                1045                1050

cgg gat gcc ctc aca gtg ggg aaa aat gag gag gat gct aaa aag tat   3521
Arg Asp Ala Leu Thr Val Gly Lys Asn Glu Glu Asp Ala Lys Lys Tyr
               1055                1060                1065

ttt ctt gat cag atc gaa gtt tgc aga gac aaa gga tgg act gtg cag   3569
Phe Leu Asp Gln Ile Glu Val Cys Arg Asp Lys Gly Trp Thr Val Gln
           1070                1075                1080

ttt aat tgg ttt cta cat ctt gtt ctt ggc atc aaa caa gga gag aaa   3617
Phe Asn Trp Phe Leu His Leu Val Leu Gly Ile Lys Gln Gly Glu Lys
       1085                1090                1095

cat tca gcc taa tactttaggc tagaatcaaa aacaagttag tgttctatgg       3669
His Ser Ala
   1100

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785 790 795 800

Lys Val Met Ala Ser Lys Lys Lys Pro Leu Trp Leu Glu Phe Lys Cys
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Ile Met Glu Ser Ile Trp Glu Thr Glu Ser Leu Asp Leu Cys Leu Leu
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Pro Tyr Gly Cys Ile Ser Thr Gly Asp Lys Ile Gly Met Ile Glu Ile
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Ala Asn Tyr Val Glu Ala Ile
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 November 2001 (15.11.2001)

PCT

(10) International Publication Number
WO 01/085986 A3

(51) International Patent Classification[7]: C12Q 1/48, A61P 19/00, 29/00, 35/00, 37/00, G01N 33/50, C12N 9/12

(21) International Application Number: PCT/US01/15065

(22) International Filing Date: 10 May 2001 (10.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/203,346 10 May 2000 (10.05.2000) US

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(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **SADHU, Chaneal** [US/US]; 903 233rd Street S.E., Bothell, WA 98021 (US).

(74) Agent: **NOLAND, Greta, E.**; Marshall, O'Toole, Gerstein, Murray & Borun, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606-6402 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
27 December 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/085986 A3

(54) Title: PHOSPHATIDYL INOSITOL 3-KINASE DELTA BINDING PARTNER

(57) Abstract: There is identified a functional interaction between the catalytic subunit of phosphatidyl inositol 3-kinase delta (P13Kδ) and SH3 domain-containing polypeptides such as LASP-1. The invention provides methods of assaying the observed interaction, methods of exploiting the interaction to identify compounds that modulate the interaction, and methods of employing such modulators in the treatment of medical disorders characterized by P13Kδactivity mediated by the interaction.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/15065

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/48 A61P19/00 A61P29/00 A61P35/00 A61P37/00
G01N33/50 C12N9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 985 589 A (CHANTRY DAVID H ET AL) 16 November 1999 (1999-11-16) cited in the application abstract claims 2,3 column 4, line 25 - line 42	19-35
Y	--- -/--	36,37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2002	10/10/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stricker, J-E

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DIDICHENKO S A ET AL: "CONSTITUTIVE ACTIVATION OF PROTEIN KINASE B AND PHOSPHORYLATION OF P47PHOX BY A MEMBRANE-TARGETED PHOSPHOINOSITIDE 3-KINASE" CURRENT BIOLOGY, CURRENT SCIENCE,, GB, vol. 6, no. 10, 1 October 1996 (1996-10-01), pages 1271-1278, XP002037800 ISSN: 0960-9822 abstract --- DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 31 March 1998 (1998-03-31) QIU YUN ET AL: "Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3'-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells." Database accession no. PREV199800230700 XP002203057 abstract & PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 7, 31 March 1998 (1998-03-31), pages 3644-3649, March 31, 1998 ISSN: 0027-8424 --- CHANTRY D ET AL: "P110DELTA, A NOVEL PHOSPHATIDYLINOSITOL 3-KINASE CATALYTIC SUBUNIT THAT ASSOCIATES WITH P85 AND IS EXPRESSED PREDOMINANTLY IN LEUKOCYTES" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 272, no. 31, 1 August 1997 (1997-08-01), pages 19236-19241, XP002059998 ISSN: 0021-9258 cited in the application abstract --- -/--	36 37 19-35

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VANHAESEBROECK B ET AL: "P110BETA, A NOVEL PHOSPHOINOSITIDE 3-KINASE IN LEUKOCYTES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 94, no. 9, 29 April 1997 (1997-04-29), pages 4330-4335, XP002044005 ISSN: 0027-8424 cited in the application abstract	19-35
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; July 1998 (1998-07) CHEW C S ET AL: "Lasp-1 is a regulated phosphoprotein within the cAMP signaling pathway in the gastric parietal cell." Database accession no. PREV199800388860 XP002202774 cited in the application abstract & AMERICAN JOURNAL OF PHYSIOLOGY, vol. 275, no. 1 PART1, July 1998 (1998-07), pages C56-C67, ISSN: 0002-9513	1-18
A	KLIPPEL A ET AL: "A REGION OF THE 85-KILODALTON (KDA) SUBUNIT OF PHOSPHATIDYLINOSITOL 3-KINASE BINDS THE 110-KDA CATALYTIC SUBUNIT IN VIVO" MOLECULAR AND CELLULAR BIOLOGY, WASHINGTON, DC, US, vol. 13, no. 9, 1 September 1993 (1993-09-01), pages 5560-5566, XP002037804 ISSN: 0270-7306 abstract	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/[redacted]01/15065

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13-18, 29-37
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 19-34 (partially), 11, 12, 27 and 28 (totally)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 13-18 and 29-37 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 13-18, 29-37

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

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Continuation of Box I.2

Claims Nos.: 19-34 (partially), 11, 12, 27 and 28 (totally)

Present claims 19-34 relate to an extremely large number of possible polypeptides comprising an SH3 domain. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found (p.36, l.10), however, for only a reduced number of polypeptides. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the polypeptides p85, p47-phox and Btk.

Present claims 11, 12, 27 and 28 relate to a substance and its use defined by reference to a desirable characteristic or property, namely an activator or inhibitor of the binding between p110 delta and an SH3 containing polypeptide, e.g. LASP-1.

The said claims cover all substances having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such substances. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the substances by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Info on patent family members

International Application No
PCT/US 01/15065

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5985589 A	16-11-1999	US 5882910 A	16-03-1999
		US 5858753 A	12-01-1999
		AU 5458798 A	22-06-1998
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